## Long-term trends in aquatic invertebrates indicate river health in England and Wales



A thesis submitted for the degree of Doctor of Philosophy

by

Emma Pharaoh

School of Biosciences Cardiff University

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#### Summary

Rivers are highly associated with human activities within the river and their surrounding catchments. Such as, hydrological alteration, land use activities and their implications on water quality. These impacts vary across space and through time. It is important that the ecological consequences of these stressors are understood to inform policy and management decisions. River invertebrate data is abundant across the UK, with invertebrate communities responsive to changes in environmental conditions. Therefore, invertebrate data is a valuable tool for assessing changes in the ecological status of rivers, investigating how communities are responding to changing conditions and inferring the overall status of rivers.

This thesis looked to identify how river invertebrate communities have changed over the last three decades (1991-2019) across England and Wales, using national monitoring data of invertebrates collected by the Environment Agency and Natural Resources Wales. This also aimed to clarify the roles played by water quality, catchment land use, climate, and hydrology in driving these changes. Analyses explored multiple facets of invertebrate diversity (e.g. community composition, family richness, functional diversity) and spatial scales, ranging from national (England and Wales) to a single catchment (river Wye).

The results of this thesis confirmed that river invertebrate communities have been changing through time, with an overall recovery throughout the time series both nationally and within the Wye. Recovery occurred in both urban and agricultural areas, with the greatest recovery occurring in urban areas. Spatially, these trends varied, with some notable areas of decline, including within the uplands. The role that environmental variables (e.g. water quality, land use) played in these trends also varied spatially, whilst interactions between water quality, hydrology and catchment variables were identified. These results highlighted the complexity and difficulty in attributing changes in invertebrate communities to environmental variables nationally and at the catchment scale.

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## Statements and Declarations

#### Statement 1

This thesis is being submitted in partial fulfilment of the requirements for the degree of PhD

#### Statement 2

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#### Declaration

This thesis is the result of my own independent work, except where otherwise stated, and the views expressed are my own. Other sources are acknowledged by explicit references. The thesis has not been edited by a third party beyond what is permitted by Cardiff University's Use of Third Party Editors by Research Degree Students Procedure.

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#### Chapter 1. General Introduction

"In every respect the valley rules the stream" Hynes, 1975

#### 1.1 Overview

Rivers and other freshwaters are hotspots of biodiversity, which despite covering just 0.8 % of the Earth's surface, support around 9.5 % of all described species, including approximately a third of vertebrates (Lehner and Döll 2004; Balian *et al.* 2008). However, freshwater biodiversity has declined substantially, including an estimated 84 % decrease in global freshwater vertebrate abundance since 1970 (Strayer and Dudgeon 2010; Collen *et al.* 2014; WWF 2020). Rivers are closely associated with human activities, such as agriculture, industry and habitation, which are often concentrated near water sources (Vörösmarty *et al.* 2010). This makes river ecosystems particularly vulnerable to overexploitation, pollution, invasive species, modification and other activities which can lead to their degradation (Dudgeon *et al.* 2006; Vörösmarty *et al.* 2010; Reid *et al.* 2019). Moreover, rivers are highly connected both within their network of channels and to the surrounding environment, enabling the easy transfer of stressors such as pollution and invasive species across watersheds (Dudgeon *et al.* 2006).

The ecological integrity of rivers is influenced by a host of physical, chemical and biological factors (Karr and Chu 2000). Their quality is partly a reflection of the activities, land use and management practices carried out within the catchment (Allan *et al.* 1997; Fierro *et al.* 2017; Nelson Mwaijengo *et al.* 2019). Rivers in urban areas are considered to be exposed to the most pressures, including changes in hydrology, geomorphology, water temperature, invasive species and water quality changes (Brown *et al.* 2005b; Chadwick *et al.* 2006; Kinouchi *et al.* 2007; Francis *et al.* 2019). Urban water quality can be affected by diffuse pollution (e.g. runoff from residential, industrial areas and roads) and point sources (e.g. sewage treatment works, industrial effluent and combined sewer overflows). Contaminants include inorganic nutrients, fine sediment, microplastics and pharmaceuticals. As with all land use activities, their impacts also vary with factors such as proximity of activities to a watercourse, topography and hydrology which can influence the ability of pollutants to reach watercourses (Dabrowski *et al.* 2002; Davey *et al.* 2020; Wang *et al.* 2020).

71 % of land area in the UK is agricultural, with agricultural and rural land management contributing to the largest number of assessments of poor water quality in the UK (DEFRA 2016; Environment Agency 2019). The implications of agricultural and rural activities on rivers is largely governed by land uses and agricultural practices (e.g. forestry, high/low arable intensity, pastoral) (Lam *et al.* 2010; Taylor *et al.* 2016; Nelson Mwaijengo *et al.* 2019). Broadly, the most prevalent pollutants from agricultural activities are deposited fine sediments and elevated nutrient concentrations (namely phosphorous and nitrogen) (Davis *et al.* 2018). Other sources of pollutants within rural areas include poorly maintained septic tanks (Withers *et al.* 2011), sewage treatment works (Neal *et al.* 2005) and historic mining activities (Beane *et al.* 2016).

Long-term monitoring of water chemistry requires regular and often costly sampling and analytical methods. In addition, due to the nature of rivers, pollutants can pass through a system quickly, which can result in pollution events being missed. Biomonitoring, the use of organisms to survey the environment, can overcome some of these challenges (Rosenberg and Resh 1993). Here, changes in an indicator species or assemblage, such as abundance, presence or behaviour, can signal changes in environmental conditions (Carignan and Villard 2002). Macroinvertebrates are widely used for monitoring the environmental condition of waterbodies, due to their diverse ecology and ease of collection (Cairns and Pratt 1993; De Pauw et al. 2006; Buss et al. 2015). They are a phylogenetically diverse group, reflected in a wide range of trophic levels, environmental requirements, and tolerances to stressors (Cook 1976; Pratt and Coler 1976). Invertebrates also have a relatively long lifespan, which combined with sensitive life stages and a largely sedentary nature, means that their presence can reflect local environmental conditions (Cook 1976; Bonada et al. 2006). Macroinvertebrates are therefore a valuable group for monitoring environmental conditions and studying responses of communities to changes in these conditions. As a result, there is an abundance of macroinvertebrate data which have been collected in the UK and globally (Bonada et al. 2006; Vaughan and Ormerod, 2012; van Klink et al. 2020). These data, which often span many years, provide a valuable resource for answering questions around the ecological status of rivers through time and the implications of stressors on this.

The condition of rivers in the UK has attracted significant public attention in recent years, in particular due to concerns that river quality may be declining (RSPB *et al.* 2021; The Rivers

Trust 2021; House of Commons Environmental Audit Committee 2022). This has been linked to ongoing point-source pollution, such as combined sewage overflows and intensive agriculture (e.g. leaks from slurry stores). Links have also been drawn with diffuse agricultural pollution (e.g. runoff of soil, manure, chemicals from agricultural land) and emerging threats such as microplastics and climate change (Surfers Against Sewage 2021; Water UK 2021). A recent national assessment of the chemical quality of British rivers has revealed a mixed picture of change through time, with some long-term improvements, while there are other areas of ongoing and increasing concern (Whelan et al. 2022). Monitoring of the biological quality of UK rivers has also been undertaken by agencies such as the Environment Agency (EA) and Natural Resources Wales (NRW), in addition to charities and researchers. Data collected can provide insights into the long-term condition of rivers (e.g. Dunbar et al. 2010; Mathers et al. 2019; Ormerod and Durance, 2009; Outhwaite et al. 2020; Powell *et al.* 2023; Vaughan and Ormerod, 2012). Macroinvertebrates have been regularly sampled across England and Wales by the EA and NRW at numerous sites. For this sampling a quality-assurance scheme ran from 1991-2009 which indicated that the error rate was near-constant (Murray-Bligh and Griffiths 2022), and similar protocols remained after this date. Therefore, and especially for macroinvertebrates, there is a large volume of highquality data available.

There is an increasing volume of research underpinned by macroinvertebrate data collected by statutory agencies. Much of this has concentrated on how trends through time have varied across space, including across different river typologies, altitudes, and catchment land use types (e.g. Vaughan and Ormerod, 2012; Powell *et al.* 2023; Qu *et al.* 2023). Some work has also aimed to identify what variables, such as flow variability or climate change, may be responsible for changes in invertebrate communities through time and space (Durance and Ormerod, 2007; Vaughan and Ormerod, 2012). These types of assessments can be challenging, due to the interconnected nature of environmental variables (Harpold *et al.* 2010; Wilkes *et al.* 2019; Birk *et al.* 2020). However, assessments of trends through time and drivers of these can provide important information for land managers and policymakers. This information can be used to identify where resources should be directed to improve the health of rivers.

#### 1.2 Project aims

The overall aims of this thesis were to identify how river invertebrate communities have changed over the last three decades (1991-2019) across England and Wales, and clarify the roles played by water quality, catchment land use, climate and hydrology. This used national monitoring data of invertebrates and water chemistry collected by the EA and NRW, along with other data from across England and Wales (e.g. land use, discharge, altitude and geology). The analyses explored multiple facets of invertebrate diversity, including community composition, family richness, functional diversity and beta-diversity, with spatial scales ranging from national (England and Wales) to a single catchment.

Chapter 2 reviews the literature surrounding the quality and status of rivers. This covers how and why macroinvertebrates are used to assess the status of waterbodies. The chapter also details river stressors, their sources and impacts on invertebrate communities, as well as reviewing the current literature of river invertebrate trends in the UK.

Chapter 3 investigates changes in river macroinvertebrate communities across England and Wales at large, utilising a large sample pool of almost 4,000 survey locations. Analysis included multiple measures of the macroinvertebrate community, including family and functional richness, community composition and turnover. The key hypothesis tested was that biological recovery occurred in the 1990s, yet stalled in the most recent 10-15 years. This chapter also explores how temporal trends varied with catchment characteristics, including land use. This tested the hypothesis that recovery will be greater and continue for longer within more heavily urbanised catchments than in rural catchments. A modified version of this chapter, with additional analyses, was published during the preparation of this thesis (Pharaoh *et al.* 2023; Appendix A).

Chapter 4 focuses on highlighting and portraying some of the complexities which arise when describing long-term trends in invertebrate communities at large scales and attributing changes to environmental variables. The chapter also demonstrates the regional variation in long-term change. It uses two relatively novel techniques: structural equation modelling investigated the interconnected relationships amongst environmental variables and invertebrate measures, whilst geographically weighted regression modelling tested the

hypothesis that relationships between invertebrate communities, and water quality and land use, will vary across space.

The river Wye is used as a case study in Chapter 5, which involves a more in-depth analysis of the long-term trends in invertebrate communities at the catchment scale. The analysis builds on the regional variation in changing river status revealed in Chapter 4 and utilises a subset of the national monitoring sites used within Chapters 3 and 4, with the same 29-year time-series. The chapter takes advantage of the smaller spatial scale to investigate links between the land cover change and invertebrate communities, and examine more intricate facets of change in invertebrate communities (taxa and traits).

Finally, the General Discussion (Chapter 6) draws together the outcomes from Chapters 2-5. This discusses how invertebrate communities have changed through time and how environmental variables have been found to interact with communities. This goes on to discuss implications of the findings and recommends future research priorities.

# Chapter 2. A literature review exploring the impacts of stressors on rivers and their biodiversity implications

#### 2.1 Summary

Freshwaters contain a disproportionately high proportion of the Earth's biodiversity, however this has been found to be declining globally - due to human activities. In this review, literature is gathered and reviewed to explore the state of global freshwaters, outline why freshwaters contain such high diversity and explore how land use relates to stressors and threats of rivers. UK freshwater policy is then described, as are the methods used to assess the status of rivers and the importance of long-term studies, specifically describing the value of river invertebrates. The specific threats and stressors of rivers are outlined in detail, describing how these relate to invertebrate communities. This concludes by outlining the current literature surrounding river invertebrate trends in the UK. The main findings of the review are that rivers are exposed to a host of threats, of which most directly relate to activities within a river's catchment. The main conclusion is that by taking advantage of environmental monitoring data already collected, it is possible to assess the status of invertebrate communities in English and Welsh rivers. Furthermore, it is important to identify how this relates to stressors which are identified in this literature review and have available data to investigate this over the long-term. The importance of studying this over the long-term is emphasised, to unpick natural variations, as well as biotic and abiotic interactions from human driven stressors.

#### 2.2 Global freshwaters

#### 2.2.1 Freshwater diversity

Freshwaters contain just 0.01 % of the Earth's water, with lakes, rivers and reservoirs covering around 2.3 % of the planet's surface area (Lehner and Döll 2004). These contain around 9.5 % of all described species and approximately a third of vertebrates, making freshwaters global biodiversity hotspots (Balian *et al.* 2008). Despite this, a global decline in freshwater biodiversity has been observed over decades (Jenkins 2003; Collen *et al.* 2014). Declines have been well documented using the Living Planet Index (LPI), which has tracked changes in populations of vertebrates from 1970 onwards. In the years 1970 to 2012 the freshwater index declined by 81 %, compared to marine and terrestrial declines of 36 %, 38

% respectively (WWF 2016). While these data just represent vertebrates, other analysis also support this trend. Collen *et al.* (2014) identified a higher threatened percentages of all freshwater groups studied (mammals, amphibians, reptiles, fishes, crabs and crayfish) than their terrestrial counterparts (Collen *et al.* 2014). It is important to note that while declines in freshwater diversity have been demonstrated, they are far less studied than terrestrial systems (Di Marco *et al.* 2017). It has been suggested that this presents a greater challenge for monitoring condition, diversity, decline and tackling stressors of freshwater systems (Reid *et al.* 2019).

#### 2.2.2 Human interactions with freshwaters

Freshwater systems and the surrounding landscapes have been highly connected to humans for millennia and are fundamental to human life. These are used for drinking water, transportation, waste disposal, power production and harvesting of resources such as, fish, plants, game and minerals. As a result, these are often the sites for homes, farms and industry. The economic value of freshwater systems is therefore vast, with these and additional ecosystem services being valued at an estimated \$4 trillion annually (Millennium Ecosystem Assessment 2005; Costanza *et al.* 2014).

Freshwaters are particularly vulnerable due to exploitation and their close human association (Dodds *et al.* 2013), with the rapid growth of the human population and global economy increasing their usage and degradation (Vörösmarty *et al.* 2010). Freshwater ecosystems are therefore at risk from a multitude of stressors that are often primarily concentrated around areas of intense agriculture, industry and domestic activity (Vörösmarty *et al.* 2010). Furthermore, these systems are particularly at risk due to the high level of connectivity within the freshwater and surrounding environment, making stressors such as pollution and invasive species transfer easily across watersheds (Dudgeon *et al.* 2006).

This has led to a whole host of threats to freshwater ecosystems, which Dudgeon *et al.* (2006) divided into five leading and persistent categories; over-exploitation, water pollution, habitat degradation, species invasion and flow modification. Other emerging threats outlined include; emerging contaminants and changing climates (Reid *et al.* 2019). Leading threats have largely remained static in the freshwater agenda over the past decade, however the understanding and prioritisation of emerging threats has been changing (Dudgeon *et al.* 

2006; Reid *et al.* 2019). For example, the increasing understanding of the impact of climate change beyond that of water temperature and flow regimes has heightened the priority of this specific stressor (Scheffers *et al.* 2016). This range of threats to freshwaters combined with the evident declines in diversity makes research into these systems vital for informed policy and management at the global, regional and local level.

#### 2.3 Rivers

#### 2.3.1 Diversity within rivers

Biological diversity in natural ecosystems is governed by the community assembly within the system. This is determined by the abiotic conditions (such as climate, geology and flow rate), biotic interactions and niche processes. These determine the suitability for individual species and the ability of a species to colonise suitable regions (Weiher et al. 2011). Natural rivers are characterised by vast levels of environmental heterogeneity (Ward 1989). Heterogeneity in habitat and environmental conditions and its influence on diversity can be observed a variety of scales. Ranging from those across entire landscapes and catchments to smaller scales such as the meso or microhabitat scale. The makeup of a river reach with its range of micro and mesohabitats leads to a range of different environmental and habitat conditions (e.g. flow, sediment, macrophytes). These heterogeneous environmental and habitat conditions would therefore influence suitability to different invertebrate communities (Garcia et al. 2012). Consequently, benthic invertebrate assemblage composition and richness are strongly correlated to the environmental variation at reach and basin level (Strayer 2006). While some rivers have more stable environmental conditions, others are naturally more dynamic or stressed (e.g. periodic drying, salinity or high elevation). These abiotic stressors frequently contribute unique invertebrate taxa to the species pool which influences alpha and beta diversity (Gutiérrez-Cánovas et al. 2013; Leigh and Datry 2017; Stubbington et al. 2019).

The ability of colonisation by species is determined by the regional species pool and the chance/ dispersal abilities of organisms to colonise a suitable site (Weiher *et al.* 2011). Lotic environments have a dendritic structure, with tributaries converging to form the main river channel which influences the dynamics of these systems (Benda *et al.* 2004). While some landscapes are more two dimensional, allowing colonisation across a variety of routes, landscape structure and physical flow of rivers can dictate the distance and direction of

dispersal (Altermatt and Fronhofer 2018). Isolation can therefore occur at fine scales, where some areas have low connectivity with the rest of the river network (Hughes *et al.* 2009), and more isolated aquatic habitats will be colonised more by winged adults in the terrestrial environment (Lancaster *et al.* 2024). For instance, arrivals of individuals in headwaters are more limited to individuals dispersing within the river network than locations downstream. This can lead to relatively low alpha diversity in individual headwater streams, but higher beta diversity among headwater streams within catchments and higher gamma diversity among catchments (Clarke *et al.* 2008). Aerial dispersers would be less influenced by flow directions, which are able to disperse overland between different branches of the river network (Tonkin *et al.* 2018; Lancaster *et al.* 2024). Interactions among these types of dispersal limitations and niche suitability therefore determines diversity across the lotic environment (Astorga *et al.* 2012).

Local communities are therefore reflective of the cumulative effect of these processes (Wiens and Donoghue 2004; HilleRisLambers *et al.* 2012). This combination of factors results in vast differences in rivers across landscapes, leading to a wide total diversity of river systems.

#### 2.4 Land use – implications for rivers

The quality of rivers and other freshwater systems is partly a reflection of the activities, land use and management practices carried out within the catchment, at various scales (Allan 2004; Fierro *et al.* 2017; Nelson Mwaijengo *et al.* 2019). As these are located amongst and downhill of terrestrial systems, the drainage basins of these influence the status of river systems. For example, hydrological conditions vary across broad vegetation types and land use types such as urban-rural gradients or agricultural practices. Here changes in hydrology occur as a direct consequence of human activities within the catchment, which can then have knock on effects on further river quality (O'Connell *et al.* 2007; Pattison and Lane 2012; Miller and Hess 2017). Put simply in the Hynes (1975) quote, '…*in every respect the valley rules the stream…*'.

Globally lotic habitats have been considered the most heavily modified freshwater ecosystems, for the purposes of flood control, navigation and the use of the river corridors (Ward and Tockner 2001). This is true of rivers in the UK, with modifications carried out over centuries and to varying degrees (Mainstone and Wheeldon 2016). The most heavily modified rivers tend to be concentrated in more urban areas and lowland fens, while the uplands contain more natural rivers (Environment Agency 2010; Environment Agency 2015a). Modifications include the re-sectioning of river lengths, such as deepening or widening channels to reduce flooding of adjacent land and in-channel structures e.g. weirs and sluices (Environment Agency 2010). The varied nature of these modifications means that the implications on river ecosystems varies (Pedersen *et al.* 2006; Mainstone and Wheeldon 2016).





While some stressors directly link to the land use within the catchment or activities carried out on rivers themselves, others relate to activities and the consequences of these from outside of a catchment. For example, climate change where although interaction among stressors within rivers and their catchments will occur, is a wider scale stressor with the potential to influence freshwaters far removed from the activities driving this process (Knouft and Ficklin 2017). Other pollutants can travel large distances from their source, such as persistent organic pollutants or acidification because of acidic deposition (Wania 2003; Menz and Seip 2004; Nøst *et al.* 2018).

#### 2.4.1 Influence of urban and rural land uses on rivers

The UK is one of the most densely populated regions in Europe, with a growing population (ONS 2019). This has resulted in many densely populated urban areas, which over 80 % of the UK's population inhabits (Bettencourt and Lobo 2016; DEFRA 2020). Rivers are considered to be exposed to the greatest pressures in urban areas, due to the pressures

associated with the land use and high population density (Brown *et al.* 2005; Walsh *et al.* 2005; Chadwick *et al.* 2006). These pressures include changes in hydrology, geomorphology, as urban rivers are heavily modified and flow dynamics are influenced by impervious surfaces. Water temperature is also influenced by concrete in urban areas (Herb *et al.* 2008; Xin and Kinouchi 2013). Water chemistry is altered through diffuse pollution (e.g. from residential and industrial runoff, construction and vehicle emissions) and point source pollution from treated discharges (e.g. sewage treatment works, industry) and untreated discharges (e.g. Combined Sewer Overflows). These are responsible for a range of contaminants, including nutrient pollution, sedimentation, microplastics, pharmaceuticals and emerging contaminants (Nelson and Booth 2002; Cristale *et al.* 2013; Miller and Hutchins 2017). Urban rivers are also often colonised readily by non-native species (Francis *et al.* 2019). These pressures can in turn impact river ecology and diversity, with many rivers described as exhibiting ecological degradation known as "urban stream syndrome" (Walsh *et al.* 2005).

Despite the significant pressures of urban areas on river water quality, in some heavily polluted catchments in the UK improvements have been observed (Langford *et al.* 2009). This has been attributed to a variety of factors, including the reductions in industrial activities (since a peak in the first half of the 20<sup>th</sup> century), legislation and developments in technology and infrastructure which can treat point sources of pollution (Langford *et al.* 2009). In more urbanised catchments, biological recovery has also been seen (Vaughan and Ormerod, 2012).

Within the UK 71 % of land area is agricultural, which can be expected to influence a vast quantity of the UK's river systems (DEFRA 2016), with intensification of agricultural practices since the Second World War (Angus *et al.* 2009). This agricultural intensification has not just changed how much land is managed but also how land is managed, which has changed the hydrology and pollutants associated with rural land (O'Connell *et al.* 2007). Partly as a result of this, agriculture and rural land management contribute to the largest number of assessments of poor water quality in the UK. This is responsible for around six times more failings than reasons related to urban activities and transport (Environment Agency 2019).

As agricultural/rural activities vary, so do the implications of these on river systems. In terms of catchment hydrology, this is influenced by vegetation type, cover and compaction which

can influence runoff, infiltration and by extension influence river hydrology (Brown *et al.* 2005a; Price 2011; Rogger *et al.* 2017). Within rural areas this can relate to activities, such as deforestation, land conversion, agricultural practices (forestry/arable/pastoral) and agricultural intensity (e.g. stocking density) (Öztürk *et al.* 2013; Marshall *et al.* 2014; Alaoui *et al.* 2018). Similarly, the pollutants entering waterways from rural areas are governed by the land uses and agricultural practices (Yong and Chen 2002; Buck *et al.* 2004; Taylor *et al.* 2016). For example, artificial nutrient additions, manure production, pesticide use, and vulnerability to soil erosion correspond to various activities and their intensities. This also varies with factors such as proximity to a watercourse, topography, hydrology and best practice or mitigation actions. These influence the ability of pollutants to reach watercourses (Dabrowski *et al.* 2002; Tournebize *et al.* 2017; Davey *et al.* 2020). Other sources of pollutants within rural areas which are less associated with agricultural land use include; poorly maintained septic tanks, sewage treatment works and historic mining activities (Neal *et al.* 2005; Withers *et al.* 2011; Beane *et al.* 2016).

Broadly, the most prevalent pollutants from agricultural activities have been identified as deposited fine sediments and elevated nutrient concentrations (namely phosphorous and nitrogen) (Davis *et al.* 2018). With improvements in the water quality of urban areas (Langford *et al.* 2009) and given that a large proportion of land within the UK is rural (DEFRA 2016), rural rivers should be characterised as an area of substantial research interest. Particularly as fine sediment and nutrient pollution, which are partly attributed to agricultural/rural activities, are considered among the most prevalent threats to rivers systems (Environment Agency 2015a; Lemm and Feld 2017; Davis *et al.* 2018; Environment Agency 2019).

#### 2.5 UK environmental schemes, legislation and policy

Within the UK, freshwaters are protected under a range of schemes, policies and legislation. One example at the UK level involves regulating and permitting of potentially environmentally damaging activities (e.g. the discharge of water from sewage treatment works), overseen by the Environmental Permitting Regulations 2010 (DEFRA 2013). Also at the UK level, the Water Act 2003 licenses abstractions by the water industry (Water Act 2003). Another example is Catchment Sensitive Farming, a voluntary scheme which works by partnering farmers and land managers with water companies, local authorities and environmental bodies to provide free training on budgeting fertiliser and pesticide use, reducing soil loss (Environment Agency 2014). Many UK environmental laws and policies were rooted in the European Union (EU), although those which are independent often work in tandem with larger scale projects (McDowell *et al.* 2016; Reid 2016). Most notably the Water Framework Directive.

The EU Water Framework Directive (WFD) was introduced in the year 2000 to establish a framework for the protection of European water bodies (European Commission 2000). This directive aimed to improve and protect water quality (groundwater, surface and coastal water) across Europe by ensuring these reach and maintain good ecological and chemical status. This mandates that member states produce planning documents which outline how good status will be achieved, with plans and updates on results carried out in six year cycles (Newig and Koontz 2014). Planning is divided at the river basin level, of which River Basin Management Plans and Programs of Measures are drawn up from. These are produced by all stakeholders in the area, with proposed documents used as the main driver of policy implementation (Kochskämper *et al.* 2016).

Central to successful implementation is the effective monitoring of water bodies, with an emphasis on ecological quality, opposed to just the physical or chemical qualities of water bodies (Logan and Furse 2002). Ecological quality is therefore assessed by both river biological measures (e.g. macroinvertebrates, macrophytes and fish) and chemistry, hydrology and morphology (Heiskanen *et al.* 2004). While member states sampling methods vary, these data can be transformed so that comparisons can be made across boundaries (Bennett *et al.* 2011). Despite praise for its ambition (Hering *et al.* 2010), there are several shortcomings limiting the successful implementation of the WFD (Voulvoulis *et al.* 2017; Berbel and Expósito 2018; De Vito *et al.* 2020).

Other European directives which influence freshwaters include the Urban Wastewater Treatment Directive, introduced to regulate the collection, treatment and discharge of wastewater from urban areas (Council of European Communities 1991). Given that much of the UK's environmental laws and policies are deeply rooted within the EU, suggestions have been made of future implications of the UK's departure from the EU (Reid 2016; Burns *et al.* 2019). However, the WFD has been retained in UK law to date.

#### 2.6 Assessing the status of rivers

#### 2.6.1 Defining rivers – rural or urban?

As urban and rural land use has different effects on the physical conditions within rivers, this must be factored in when investigating these systems. The terms urban and rural are used by a wide range of policymakers, researchers, governments, and international organisations. As a result the categorisation and determination of land type is based on a range of measures e.g. land cover, population density and cultural practices (Halfacree 1995; Pateman 2011; Office for National Statistics 2013; Dijkstra and Poelman 2014; Ratcliffe *et al.* 2016). The European Union defines rural areas as those outside of urban clusters, dividing urban areas into two categories. Urban centres (or "high-density clusters") are adjacent 1 km<sup>2</sup> areas with a density of at least 1500 inhabitants per km<sup>2</sup> and a minimum population of 50,000. While "urban clusters" are clusters of adjacent grid cells of 1 km<sup>2</sup> with a density of at least 300 inhabitants per km<sup>2</sup> and a minimum population of 20 hectares spread (< 200 metres spacing) combined to form one built up area with a population of at least 10,000 usual residents". Areas with fewer than 10,000 residents are then classed as rural (Pateman 2011; Office for National Statistics 2013).

Similarly, in the scientific literature the definition of rural or urban areas in the UK varies, both within and across disciplines. Examples of this are presented in Table 2.1, which shows across a range of study scales the varying use of definitions or measures to determine whether the river/s studied are urban or rural. Here some studies do not define how the areas of study are determined as rural or urban, with some using the term "rural" synonymously with "agricultural" or defining rivers based as how rural or urban they are comparatively. Others, including studies at larger landscape and catchment scales use land cover/use data or population density data to classify rivers.

Literature	Definition/ urban-rural distinguishing measure	Study scale
McGrane,	CORINE 2000 European Land Cover Database and	Multiple catchments, North-East
Tetzlaff and	human population within catchment	Scotland
Soulsby (2014)		
Hutchins (2012)	CEH Land Cover Map, 2000 and DEFRA Agricultural	Catchment (River Ouse, North
	Census data	Yorkshire)
Jarvie <i>et al.</i>	Rural areas categorised further with agricultural	2 catchments (Wye, South Wales
(2008)	type, e.g. arable. Population density included.	and South West England and Avon,
		South England) and rivers within
		these.
Vane <i>et al.</i>	Rural or urban areas not specifically defined, sites	River/catchment scale (River Clyde,
(2019)	are described as being more rural, urban (Glasgow)	Scotland)
	and estuarine areas.	
Tibbetts <i>et al.</i>	Population density of local districts 3km upstream	Catchment (River Tame,
(2018)	of sampling location.	Birmingham)
Tye <i>et al.</i>	Comparatively more urban/rural areas sampled.	Catchment (River Nene,
(2016)		Northamptonshire)

Table 2.1 Measures and definitions of "rural" rivers in the UK within the scientific literature.

Land use datasets which have been used in a few of these studies also use varying definitions, divisions of land types and methods of data collection (Table 2.1). The CORINE Land Cover Database provides information on land cover and use for the European Union, combining satellite data with other available data sources such as population census, agricultural production data, forest inventories and aerial photographs (Büttner *et al.* 2017; European Environment Agency 2017). This divides land across three tiers, totalling 44 categories at the 1 km scale (Cole *et al.* 2018). The CEH Land Cover Map provides land cover information across the UK, to as much as 25 m resolution. Satellite data are used to classify land into 21 categories based on the UK Biodiversity Action Plan Broad Habitat definitions (Jackson 2000). Both these databases have different land classifications categories and cut offs between each category (Table 2.2). This on top of the differences in approaches with combining the satellite data with other data types, such as aerial photographs, could lead to different data classifications even if the same categories were used (Wentz *et al.* 2006; Sertel *et al.* 2018). These differences in approach have the potential to result in variations in

land classed as rural. For example, the CORINE dataset divides urban areas into "Continuous urban fabric" and "Discontinuous urban fabric" whereas the CEH Land Cover Map divides built-up areas and gardens into "Urban" or" Suburban categories".

Table 2.2 Classification of rural and urban areas within CORINE Land Cover Database and CEH Land Cover Pap.

Dataset/land	Example of rural/urban areas classification			
cover map				
		Tier 1	Tier 2	Tier 3
CORINE	Rural	Agricultural land	Arable land	Non- irrigated
				arable land
	Urban	Artificial	Urban fabric	Continuous urban
				fabric
		Aggregate Class	Broad Habitat	Target class
CEH Land	Rural	Arable	Arable and	Arable and
Cover Map			horticulture	horticulture
	Urban	Built-up areas and	Built up areas and	Urban
		gardens	gardens	

It is important to consider that rivers, by their nature, will pass through a range of land uses from their headwaters to the mouth. This makes determining whether a river is "rural" or "urban" difficult, as river catchments can encompass a range of different land uses and habitat types. A range of studies have used rural-urban gradients to account for this (Urban *et al.* 2006; Clapcott *et al.* 2012; Vaughan and Ormerod, 2012). For the purpose of this research, and due to the availability of a large dataset spanning across the UK, survey locations will be classed as rural according to the land cover of the catchments for individual sites. Rivers will be excluded from analysis which are fed by catchments above that of a predefined coverage of urban land cover class, using the CEH Land Cover Map.

#### 2.6.2 Biomonitoring and bioassessments

A host of physical, chemical and biological factors influence the ecological integrity of river ecosystems (Karr and Chu 2000). Long-term measuring and monitoring of these require regular, intense and costly sample methods. Biomonitoring, the use of biological variables to survey the environment, can bypass some of these costs (Rosenberg and Resh 1993). Biomonitoring requires an indicator (a species or assemblage) with a specific requirement for a known set of environmental conditions. Changes in presence, abundance or behaviour of such an indicator can signal a change in environmental conditions (Carignan and Villard 2002). Various biota have been used to monitor aquatic ecosystems, including algae, macrophytes, macroinvertebrates, fish and birds (Rosenberg and Resh 1993; Bryce et al. 2002; Pont et al. 2006; Wu et al. 2017). For the management and conservation of freshwater resources it is critical for changes in natural and anthropogenic stressors through time to be measured and monitored effectively, for which biomonitoring is widely used for (Friberg et al. 2011). Macroinvertebrates are among the most frequently used group for monitoring water body environmental condition, partly due to their ecology and ease of collection (Cairns and Pratt 1993; Bonada et al. 2006; De Pauw et al. 2006). They are used to assess conditions ranging from stream to continental scales (Hawkins et al. 2000; Haase et al. 2023). While not a taxonomic concept, macroinvertebrates are widely defined as organisms which can be caught or retained in a vessel with a mesh size of  $250 - 1000 \,\mu\text{m}$ and seen with the naked eye (with these mostly >1mm) (Rosenberg and Resh 1993).

Macroinvertebrates are ubiquitous and abundant throughout the whole river system, able to inhabit areas where other species may be unable. Their largely sedentary nature makes their presence indicative of local environmental conditions (Cook 1976), alongside biotic interactions and dispersal-related effects (Heino 2013). They have a relatively long lifespan, which combined with relatively sensitive life stages can demonstrate long-term environmental variations (Bonada *et al.* 2006). The diversity of macroinvertebrates, in terms of systematics and phylogeny, results in a wide range in trophic levels, environmental requirements and tolerances to anthropogenic stressors e.g. acidification, fine sediment inputs and physical changes (Cook 1976; Pratt and Coler 1976). This is particularly useful when studying responses of communities and individuals to changes in environmental conditions due to the graded nature of responses, which can also be used to interpret the effects of multiple stressors on a system. Macroinvertebrates also bioaccumulate potential pollutants, and so can be used to detect pollutants at levels which may be undetectable in water (Cid *et al.* 2010; Caador *et al.* 2012).

Logistically, macroinvertebrates are relatively easy to collect and identify (Cook 1976; Bonada *et al.* 2006). Benthic macroinvertebrates are sampled using a variety of quantitative and qualitative methods, with specific protocols dependent on the function of the invertebrate data to be gathered (Everall *et al.* 2017). Invertebrates in rivers are most commonly sampled using a semi-quantitative kick sampling method, with a standardised sampling effort (time) (Bennett *et al.* 2011; Feeley *et al.* 2012; Murray-Bligh and Griffiths 2022).

#### 2.6.3 Methodologies of invertebrate biomonitoring

The long history of aquatic invertebrate biomonitoring has resulted in a diverse array of methodologies and techniques, which vary in application and effectiveness (Li et al. 2010; Friberg et al. 2011; Buss et al. 2015). These include community based biotic indices, which are used to estimate water quality by assessing the community of invertebrates and relative abundances in relation to the tolerance or sensitivity of the community to water quality parameters. Other indices include those with are stressor specific, used to identify specific stressors within a waterbody. These are summarised in table 2.3. Other methods include multimetric approaches, which generally require a reference condition to compare any changes to, which is a challenge particularly in Europe where human impacts leave virtually no pristine ecosystems (Hladyz et al. 2011b; Hladyz et al. 2011a). Determining reference conditions is said to be more challenging in lotic systems, as unlike standing waters palaeoecologically techniques cannot be used to reconstruct reference communities prior to widespread human influence on community compositions (Bennion et al. 2004; Nijboer et al. 2004). Instead biotic indices such as the River InVertebrate Prediction And Classification System (RIVPACS) use the most pristine sites available as reference sites, to represent the range of streams present in a region (Wright *et al.* 2000; Chessman *et al.* 2008). This multivariate approach is a statistically based tool where the macroinvertebrate species assemblage predicted at a given site (based on the environmental conditions of reference sites) are compared to the observed assemblage which is used to report the environmental and ecological quality of the river (Clarke and Davy-Bowker 2014).

Table 2.3 Macroinvertebrate river biomonitoring indices, including community based biotic indices and stressor specific indicators.

	Parameter	Description	
Commu	nity based biotic indices		
EPT	Ephemeroptera, Plecoptera, Trichoptera Abundance/Diversity	Estimates water quality by the relative abundance or diversity of three major orders of stream insects (Ephemeroptera, Plecoptera and Trichoptera) that have low tolerances to water pollution.	
BMWP	Biological Monitoring Working Party Score System	Individual macroinvertebrate families are ranked according to their sensitivity to water quality. The sum of all families collected in a sample is the BMWP score (Armitage <i>et al.</i> 1983).	
ASPT	Average Score per Taxon	Combined sums of families (as with BMWP), but accounts for abundance by dividing this by the number of scoring families (Armitage <i>et al</i> . 1983; Abbasi and Abbasi 2011).	
WHPT	Walley, Hawkes, Paisley and Trigg	Combined sum of families (as with BMWP), but scores are dependent on the abundance of each scoring family (Paisley <i>et al</i> . 2014)	
Stressor	specific indicators		
PSI	Proportion of Sediment- sensitive Invertebrates	Assess fine sediment within a waterbody, by calculating the percentage of sediment sensitive taxa in a sample (Extence <i>et al</i> . 2017).	
TRPI	Total Reactive Phosphorous Index	Assesses phosphorus in waterbody by calculating the proportion of phosphorous tolerant and intolerant macroinvertebrates at family level, given the river type and season (Everall <i>et al.</i> 2019)	
LIFE	Lotic-invertebrate Index for	Assess flow stress using the proportion of flow sensitive	
	Flow Evaluation	invertebrates at species level (Extence et al. 1999).	
S	Saprobic Index	The weighted average sensitivity of the invertebrate species community to organic pollution (Reynoldson and Metcalfe-Smith 1992)	

This range of biomonitoring and bioassessment tools and the relative ease of collection has resulted in a vast array of macroinvertebrate data spanning years, which has been collected by a variety of stakeholders in the UK and further afield (van Klink *et al.* 2020). This lends itself well to investigating large-scale, long-term trends in biodiversity, which when paired with environmental monitoring data can be used to assess the drivers of biodiversity trends.

#### 2.7 Long-term studies

Long-term studies are particularly important in ecology, contributing to the understanding of interannual variations/cycles, biotic and abiotic interactions, as well as disturbance and recovery following changes in natural or anthropogenic conditions (Jackson and Füreder 2006). The duration of a study contributes significantly to its conclusions, due to the dynamic nature of environmental conditions. This can result in observations which span across several days or month differing from those over years or decades (Weatherhead 1986; Elliott 1990). Longer term studies can also unpick drivers which may result in biotic or abiotic changes other than those directly studied. For example, picking apart seasonal and climatic variations (e.g. North Atlantic Oscillation) from directional climate changes (Durance and Ormerod 2007). Evidence-based policies can also be supported using long-term studies, as data and understanding at larger scales can be used in informed decision making and ecosystem management (Sutherland *et al.* 2004; Worrall *et al.* 2009; Lindenmayer *et al.* 2012). Lags in responses to stressors or changes in management and policy can also be detected in longer term studies, which would otherwise be overlooked (Burt *et al.* 2010).

There is no consensus regarding the specific time period needed for a study to be considered long-term. This can be based upon ecological criteria (e.g. time scales of ecological processes) or constraints which may be operational (e.g. human and academic lifespans) (Knapp *et al.* 2012; Lindenmayer *et al.* 2012). It is also important to consider that there is greater relative difficulty securing funding for longer term projects, which take longer to provide results (Elliott 1990). This combined with the specificity of questions puts limitations on the availability of useable long-term data. An increasing trend in meta-analysis, data mining and modelling based research is enabling powerful analysis to produce results rapidly and cheaply (Cadotte *et al.* 2012). Lindenmayer *et al.* (2012) raised concerns

that this increase could lead to reduced incentive for funding of field based long-term studies, reducing the future data available for this type of research.

In spite of this, the UK's rivers have been extensively studied and are recognised as being among the most intensively studied rivers in the world (Vaughan and Ormerod, 2012). For example, hosting long-term watershed research areas such as Plynlimon in Wales where an initial study drew in further experimental infrastructure and research (Robinson et al. 2013). This has been referred to as a "gold standard", through the process of answering an initial research question leading to policy change, further research and the collection of long-term high-quality data (Tetzlaff et al. 2017). Additionally, within the UK benthic macroinvertebrate and water quality data are collected by agencies such as the Environment Agency and Natural Resources Wales spanning a large number of sites. These reliable macroinvertebrate data date back to 1991, where quality assurance has been carried out and samples have maintained a low error rate (Vaughan and Ormerod, 2012). This data has been used in a variety of studies, were available water quality and invertebrate data can be combined with other data sources to investigate trends in macroinvertebrate communities, environmental conditions and their interactions. Studies range from smaller local and regional scales (Durance and Ormerod 2009; Langford et al. 2009; Clews and Ormerod 2010), to national (Dunbar et al. 2010a; Vaughan and Ormerod 2014a; Powell et al. 2023) and European studies (Haase et al. 2023; Sinclair et al. 2024). This provides a valuable resource for studying the effects of multiple stressors, due to the long time series and a large-scale nature of the data (Vaughan and Gotelli 2019).

#### 2.8 River stressors and threats

The stressors and threats of rivers briefly mentioned earlier require deeper discussion within a UK context. Analysis of the interactions with the physical processes and ecology of river systems is also required to develop a fuller picture. While these have been divided earlier in terms of source (Figure 2.1), the overlaps of these sources across stressors requires that these be discussed otherwise. These will therefore be divided in terms of five major categories: pollutants, river modifications, invasive species, climate change and emerging threats. These threats vary in applicability to specifically rural rivers and rivers across the board, summarised in Table 2.4.
Table 2.4 Stressors and threats of rural rivers and their implications on freshwater invertebrates. Current severity of effects is categorised as such: severe (red), moderate (yellow), fair (green) and uncertain (grey). Strength of knowledge range from: good (green), fair (yellow), poor (red). Trends that have been established as part of this review are: increasing ( $\uparrow$ ), decreasing ( $\downarrow$ ), constant ( $\rightarrow$ ), increasing and decreasing in different areas ( $\updownarrow$ ) and uncertain (?). References are in the following section of this literature review. PPCPs = Pharmaceuticals and personal care products

Stressor	Current severity of effects	Implications on rivers and invertebrates	Strength of knowledge	Trend
Nutrient enrichment	Negative relationship between nutrient concentration and diversity.	Physiochemical changes to river water, changes in habitat. Ecological regime shifts, reduced biodiversity.	Understood, but most research conducted on lentic systems.	\$
Fine Sediment	Identified by many studies as one of the major stressors of rivers.	Catastrophic drift, changes in habitat (losses of refugia), changing feeding efficiency and predation risk.	Well understood implications in river systems. Need for larger scale studies.	?
Heavy metals	Generally, more localised effects. Often sites contaminated over long time periods.	Changes in community composition, reduced diversity.	Well understood impact of metals on rivers and communities.	Ļ
Acidification	Reduced threat due to legislation. But evidence of patchy recovery.	Reduced diversity and abundance of invertebrates. Losses of acid sensitive taxa.	Well understood trends and implications in the UK.	Ļ
Pesticides	Reducing volume used but application area increasing.	Reduces diversity, implications on leaf litter breakdown.	Well understood, but research needed on "benign" levels and new pesticides.	ſ
POPs	Widespread in the environment at low levels, high risk to environment.	Bioaccumulation, endocrine disruption, neurotoxicity.	Understood but lacking research on biodiversity implications.	$\rightarrow$
Invasive species	Considered a major threat to global freshwaters.	Spread of disease, outcompeting native species.	Well understood, although specific to each species.	Ţ
River modifications (physical and flow)	Contextualise other stressors as many modifications are constant.	Implications on community structure, trophic webs, energy flows and river physiochemical conditions.	Implications and current status well understood.	$\rightarrow$
Climate change	Warming trends observed in rivers, implications assumed to be widespread.	Wide ranging implications on river physiochemistry and ecology (e.g. growth, range, phenology)	Understood reasonably. Unpredictability due to stressor interactions.	1
Emerging contaminants	Uncertainty around how biodiversity will be affected.	Microplastics, PPCP – feeding, growth and reproductive behaviour? Nanomaterials – toxicity, accumulation?	Lacking research on river concentrations and ecological implications.	1

#### 2.8.1 Pollutants

Pollutants which reach and influence rivers include: nutrients, fine sediments, metals, acid, pesticides, persistent organic pollutants (in addition to lesser known emerging contaminants which will be discussed in a later section). Whilst some of these pollutants impact freshwaters adjacent to where they are released, others are more long-range (Menz and Seip 2004). These more transient pollutants often influence watercourses irrespective of whether these are more characteristically urban or rural (Wania 2003).

#### 2.8.1.1 Nutrient enrichment

Worldwide, eutrophication is regarded as one of the most serious problems facing river ecology (Mainstone and Parr 2002; Tiwari and Pal 2022). Broadly, this is driven by an intensification of agriculture and urbanisation increasing nutrient additions to waterbodies (Cordell *et al.* 2009; Withers *et al.* 2014). Nutrients including nitrogen and phosphorous enter waterbodies from a variety of sources, dependent on the activities and land use within a waterbody's catchment. Point sources of nutrient pollution include sewage treatment works and septic tanks (Jarvie *et al.* 2006; Withers *et al.* 2011). Diffuse sources of nutrients include, inorganic and organic fertilisers applied to agricultural land which often enter watercourses in larger quantities during periods of high rainfall (Hart *et al.* 2004). The bioavailability of nutrients impacts how readily these can be taken up by primary producers, which is dependent upon the sources of nutrients. For example, inorganic nutrients from artificial fertilisers are more bioavailable than organic materials such as livestock manure, which alters the severity of effects and implications as a result (Reynolds and Davies 2001; Mainstone and Parr 2002).

Phosphorous is widely regarded as the major nutrient leading to eutrophication of UK freshwaters, as this is the limiting nutrient in rivers (Mainstone and Parr 2002; Hilton *et al.* 2006; Leaf 2018). In the UK, the main sources of phosphorous entering rivers are sewage effluent and agricultural runoff, with up to 70 % attributed to sewage discharges (Bowes *et al.* 2015; Environment Agency 2015b). Phosphorous is as a major contributor to failings of the WFD, with more water bodies in England failing to achieve good status due to phosphorus than any other WFD water quality measure. Within England around 55 % of rivers failed to achieve good status for phosphorus concentrations in 2015, with 25 % characterised as very certain of a eutrophication problem (Environment Agency 2019).

Phosphorous concentrations in rivers have been reducing since 1990, as a result of reduced phosphorous contributions from detergents, more advanced phosphorus removal at sewage treatment works, falling fertiliser use, livestock numbers and policy drivers such as Catchment Sensitive Farming initiatives (Mainstone *et al.* 2008; Environment Agency 2015b; Environment Agency 2019). In terms of reductions of phosphorus from sewage discharge, there are disparities in trends between urban and rural areas. Urban sewage treatment works tend to host more advanced phosphorous removal technology to ensure compliance with the European Urban Wastewater Treatment Directive, which has led to reductions in phosphorous output by around 80 % (Council of European Communities 1991; Bowes et al. 2005). In more rural areas where sewage treatment works are present, sewage is often treated at more basic facilities (Mainstone and Parr 2002; Neal et al. 2005). Septic tank systems are also widely used in rural areas, these should pose little threat to the environment. Evidence does, however, suggest that these are often incorrectly sited and poorly maintained (May et al. 2015). Although these systems account for a relatively small volume of waste compared to urban areas, the number of septic tanks across the UK is thought to be an underestimate, making the volume of phosphorous release within rural areas potentially significant (Dudley and May 2007; Yates et al. 2019).

Although nitrogen pollution is considered to have fewer ecological implications than phosphorus, as this is not the limiting nutrient in rivers (Leaf 2018), agricultural land is the major source of nitrate pollution within the UK. In rural catchments, agriculture is thought to be responsible for >90 % of stream and river water nitrate (Hunt *et al.* 2004; Burt *et al.* 2011). Other sources include the water industry and atmospheric emissions from industry, road transport and power generation (Environment Agency 2021a). Despite legislation, such as designation of Nitrate Vulnerable Zones (NVZs), nitrate concentrations remain high and have been increasing in UK rivers (Worrall *et al.* 2016; Environmental Audit Committee 2018). There is also evidence of increasing concentration of nitrate within groundwater, due to historic nitrate over applications which poses a particular threat to groundwater fed rivers in the UK (Wang *et al.* 2012a; Wang *et al.* 2016a; Wang and Burke 2017).

The effects of eutrophication on the structure and function of freshwater systems are relatively well understood, however within rivers this has tended to be a less researched area comparative to coastal areas and lakes (Hilton *et al.* 2006; Wurtsbaugh *et al.* 2019).

Furthermore, identification of ecological responses to nutrient pollution are difficult to detach from other river stressors due to the interaction across all trophic levels, lagged responses and ecological differences across river types (Jarvie et al. 2013; Emelko et al. 2016). Broadly, nutrient enrichment in rivers leads to excessive plant and algal growth and therefore changes in water quality measures, resulting in ecological regime shifts and ultimately a reduction in biodiversity (Smith 2003; Hilton et al. 2006; Smith et al. 2006). For instance, eutrophication can lead to changes in other water quality measures, such as dissolved oxygen (Khan and Ansari 2005). Increased plant and algae growth can shade other photosynthesising organisms which leads to deaths of algae and macrophytes lower in the water column. Resulting decomposition of these organic materials reduces oxygen concentrations (Hilton et al. 2006). This may be enhanced during the night due to a higher respiratory oxygen demand by the increased vegetation, which can influence the macroinvertebrate community (Parr and Mason 2004; Khan and Ansari 2005). While rivers tend to be more resistant than lakes due to turbulence minimising anoxia, slower flowing rivers are more vulnerable to reduced dissolved oxygen as a result of eutrophication (Dodds 2006).

Macroinvertebrate and fish measures have been seen to correlate with nutrient concentrations, implying that concentrations influence primary and secondary consumers both directly and indirectly through changes in food webs (Peterson *et al.* 1993; Yang and Sykes 1998; Wang *et al.* 2007). Changes in habitat due to increases in periphyton abundance and algal biomass can result in broad changes in macroinvertebrate diversity, e.g. increases in grazing macroinvertebrates (Tonkin *et al.* 2014). Other changes include those driven by reduced invertebrate drift (Kerans 1996), changes in food quality (Hayward and Margraf 1987) and changes in foraging efficiency (Power 1990). Elevated nutrient levels have also been shown in some cases to decrease the growth rate of certain organisms (Boersma and Elser 2006; Evans-White *et al.* 2009; Halvorson *et al.* 2015). It is widely considered that nutrient enrichment can reduce faunal biodiversity within lotic systems (Smith 2003; Hilton *et al.* 2006; Bini *et al.* 2014), although riverine community responses to nutrient enrichment can be unpredictable and variable across both fish, invertebrates and biotic integrity (Miltner and Rankin 1998; Piggott *et al.* 2012). Literature reviewed by Weijters *et al.* (2009) found that the majority of the studies investigating the relationship

between nutrient concentration and river taxon diversity have found a negative relationship. In European upland and lowland streams it has been suggested that the macroinvertebrate community as a whole responds negatively to an increasing nutrient gradient (Hering *et al.* 2006). Cook *et al.* (2018) found that eutrophication led to communities which were more temporally homogenous, driven by reductions in temporal and spatial beta diversity where there are losses in some taxa and expansion by generalists.

Climate change is predicted to increase eutrophication risks in rivers, due to potentially higher pollution concentration and excessive algal growth due to a reduction in summer flows and higher water temperatures (Johnson *et al.* 2009; Moss 2011; Hutchins *et al.* 2016). Charlton *et al.* (2018) projected that by 2050 there will be small but inconsistent increase in phosphorus concentration within English rivers. Particularly highlighting the impact of reduced dilution of effluent entering rivers from sewage treatment works, even in scenarios where there is a marked improvement in phosphorous removal.

## 2.8.1.2 Fine sediment

Fine sediment, which can be organic or inorganic particles of less than 2 mm diameter, are natural components of river systems. These enter waterbodies through erosion and deposition, and are both formed (e.g. faecal particles, phytoplankton) and processed (e.g. breakdown of detritus) by aquatic biota (Wharton *et al.* 2006; Trimmer *et al.* 2009). Due to human activities, fine sediment exceeds pre-industrial levels in many of the world's rivers (Walling and Fang 2003). Sediment loads are influenced by the intensity and scale of activities, which influence the quantity of fine sediments which enter waterbodies. This is also influenced by catchments characteristics such as topography, soil type, ground cover, riparian buffer zones and location of activities from watercourses (Wood and Armitage 1997; Ankers *et al.* 2003). Activities demonstrated to increase sediment loads to freshwaters include; agricultural land use (Lenat and Crawford 1994; Harding *et al.* 1999; Schriever *et al.* 2007), forestry/ deforestation (MacKenzie 2008), road construction (Hedrick *et al.* 2010) and mining (Hayer and Irwin 2008).

The impacts of elevated fine sediment loads on macroinvertebrates have been well observed, driven by its ecological and physiological consequences (Wood and Armitage 1997; Jones *et al.* 2012). For example, elevated loads can lead to catastrophic drift, burial, behavioural responses to protect sensitive body parts and retreat (Culp *et al.* 1986; Wood *et* 

*al.* 2001; Wood *et al.* 2005). Filter feeder efficiency can also be reduced where there are higher fine sediments, as organisms must spend more time expelling and removing unwanted particles (Strand and Merritt 1997). Increased fine sediment load can also reduce refuges available to invertebrates, as finer sediments fill in gaps between larger particles otherwise used as shelter from predators and higher flows (McClelland and Brusven 1980; Lancaster and Hildrew 1993). Thus, these and other factors can lead to changes in macroinvertebrate assemblages and changes to mesoscale habitat patches and macrophyte composition (Culp *et al.* 1983; Angradi 1999; Kaller and Hartman 2004). Macrophyte changes can in turn lead to changes in habitat, food availability, predation risk and food webs (Sand-Jensen and Borum 1991; Diehl and Kornijów 1998; Larsen *et al.* 2011).

In terms of physiological consequences, fine sediment loading to the riverbed can alter oxygen concentration within the bed, due to reduced percolation (Pretty *et al.* 2006). Decomposition of organic fine sediment can further reduce oxygen availability, leading to build up of potentially toxic substances such as ammonium (Jones *et al.* 2015). As invertebrates have varying tolerances to oxygen depletion and chemical changes, this has been shown to reflect in invertebrate assemblages and lead to reduced diversity, abundance and shifting community structure (Rasmussen 1988; Ryan 1991; Swan and Palmer 2000). Fine sediments also interact with other stressors of rivers, such as nutrients, metals, organic compounds and antibiotics, which can desorb from sediments and bioaccumulate within organisms (Wagenhoff *et al.* 2011; Zhou *et al.* 2011).

In a range of studies investigating the impacts of major stressors of invertebrates, fine sediment has been recorded as one of, and if not the most pervasive stressors of macroinvertebrate communities in lotic systems (Wagenhoff *et al.* 2011; Lemm and Feld 2017; Davis *et al.* 2018). Fine sediment has also been seen to be a major contributor to waterbodies failing to achieve good ecological status, in England being the sixth most common cause (Environment Agency 2015a). While there is considered a good biological understanding of the relationships between fine sediments and invertebrates, limited (Mondon *et al.* 2021). While much of the understanding of biological impacts of fine sediments relate to sediment deposition, most monitoring is related to suspended sediments. While an excess of fine deposited sediments has been acknowledged as a

substantial stressor affecting river ecosystems, there is still limited knowledge for management and monitoring purposes (European Environment Agency 2018b). Scale of experimental conditions across these studies have largely been focused at the mesohabitat scale, making this difficult to project to a river basin scale for management purposes (Larsen *et al.* 2009).

## 2.8.1.3 Metals

Heavy metals, such as arsenic, lead, cadmium and mercury are naturally present as trace elements in aquatic environments. Within unimpacted environments these are generally at low concentrations, entering waterbodies naturally due to the natural minerology and weathering of rocks (Karbassi et al. 2008). Anthropogenic activities which can lead to higher concentrations include; mining, agriculture, heavy industry, wastewater and sewage treatment (Nriagu 1996; Pirrie et al. 2003; Rainbow et al. 2011; Malcolm et al. 2012; Defarge et al. 2018). The intensity and regulation of these activities have varied over time, with legislation within the UK restricting some activities which can lead to environmental and human heavy metal exposure (The Council of the European Communities 1986; European Parliament 2010; Lopes et al. 2011). As heavy metals are persistent, once within aquatic bodies metals can persist and bioaccumulate (Gupta et al. 2009; Santoro et al. 2009; Protano et al. 2014). These can adsorb to suspended particulate matter, which can transfer to and accumulate in bottom sediments. Sediments can then act as reservoirs of heavy metals, leading to high concentrations which can be harmful to humans and other organisms (Pirrie et al. 2003; Rainbow et al. 2011; Protano et al. 2014). Heavy metals can then be re-released into the water column following perturbations to the sediment, such as dredging, boat traffic, fluvial erosion, bioturbation of sediments by macrobenthos and flooding events (Zhao et al. 1999; Huang et al. 2017).

Despite most mining activities within the UK having ceased, these continue to have an impact on river water quality (Beane *et al.* 2016). Elevated levels of toxic elements in the adjacent soils and rivers to mining operations have been observed, particularly in cases where activities have ceased but lack adequate remediation efforts (Hudson-Edwards *et al.* 1996; Pirrie *et al.* 2003; Rieuwerts *et al.* 2014). Heavy metals can enter water from abandoned mines from point sources such as mine adits (tunnels for drainage and working of mines) or from diffuse sources where rainwater percolates through spoil heaps and

metals enter water as dissolved particulates or are absorbed to sediments (Jarvis et al. 2006). Agricultural activities also contribute to metal contamination, where the practice of spreading sewage sludge and livestock manure can increase heavy metals within agricultural soils (Lipoth and Schoenau 2007; Madrid et al. 2007; Lopes et al. 2011). Although there are regulations on the quantities of metals added to animal feed and metal within sludge permitted for agricultural use, these can reach rural rivers from surrounding agricultural areas through leaching and surface runoff (The Council of the European Communities 1986; Shi et al. 2018; Shi et al. 2019). Whereas pesticides, herbicides and inorganic fertilisers are a source of metals elsewhere in the world, there are restrictions on the presence of heavy metals within these in the UK (Nicholson et al. 2003; Defarge et al. 2018). Other sources in the UK include emissions from waste incineration, transport, energy production and heavy industries (e.g. metal refining) (Nriagu 1990). These are then deposited in the environment, often in more rural areas, such as the uplands and forested areas of the UK (Malcolm et al. 2012). Bioavailability of metals is influenced by other factors which can influence freshwaters, such as pH, hardness of water and dissolved organic carbon which can influence the severity of metal impact on ecosystems (Jones *et al.* 2019).

Bioaccumulation of heavy metals has been well described in river invertebrates (Santoro *et al.* 2009; Kaonga *et al.* 2010; Deforest and Meyer 2015). Deposit feeders are quoted as receiving some of the highest concentrations of heavy metals, due to their feeding strategy resulting in the ingestion of fine sediment matter (Rainbow *et al.* 2009; Santoro *et al.* 2009; Casado-Martinez *et al.* 2010). River invertebrates, and functional feeding groups, show varying tolerances to heavy metals (Clements *et al.* 2000). This has been shown to cause changes in community composition, both within the UK and further afield (Clements *et al.* 2000; Armitage *et al.* 2007; Beane *et al.* 2016; Takeshita *et al.* 2019). This is largely due to decreased invertebrate diversity to comparable areas lacking metal pollution (Armitage *et al.* 2007; Santoro *et al.* 2009). Invertebrates have proven to be effective biomonitors to assess metal pollution and its bioavailability, due to their varying tolerances and similarity in responses across continents (Iwasaki and Ormerod 2012; Awrahman *et al.* 2016). The persistence and therefore continuous impact of heavy metal pollution on river communities is a characteristic of this pollutant, demonstrated in long-term studies of river invertebrates (Jones 1940; Brooker and Morris 1980; Armitage *et al.* 2007).

#### 2.8.1.4 Acidification

Acid deposition became a widespread environmental concern in the 1970s, particularly due to its impact on base poor rivers and lakes (Menz and Seip 2004). Acid deposition is caused by deposition of nitrogen oxides (from mobile sources, industrial sources and power plants) and sulphur dioxide (principally from fossil-fuel power stations, metal smelters and other stationary sources). This leads to decreased rainfall pH and increased sulphate and nitrate deposition. Areas mostly affected by acid deposition include Europe, eastern North America and Southeast Asia (Kuylenstierna et al. 2001). Land cover can play a role on the acidification of freshwater systems, both as a standalone acidifier or when combined with acid deposition (Nisbet and Evans 2014). Coniferous plantations have been heavily linked to acidification, predominantly due to scavenging of acid deposition. Here forest canopies, particularly at high altitudes, capture atmospheric sulphur and nitrogen pollutants in gaseous, particulate and cloud water forms (Fowler et al. 1989; Nisbet et al. 1995). Compared to moorland, coniferous forests increase the deposition of nitrogen oxides by an average of 106 % and the recovery of these from acid deposition has tended to be slower than that of moorlands (Kernan et al. 2010; Nisbet and Evans 2014). Furthermore, some areas are more vulnerable to acidification. In the UK this is predominantly in the uplands where the inputs of acid pollutants exceed the buffering capacity of soils and underlying rocks. This is due to base poor, slow weathering soils and high levels of deposition (Nisbet and Evans 2014). The impact of drought also results in a lowering pH of water leaving wetlands, with climate change induced drought likely to increase this (Aherne et al. 2006; Sommer and Horwitz 2009). As forestry, uplands and wetlands are characteristic rural areas which makes this a predominant problem of rural rivers.

Acidification of freshwater systems had been recorded impacting numerous organisms, ranging from macrophytes, algae, microorganisms, invertebrates and fish which leads to changes in the ecosystems and biodiversity within freshwater systems (Moiseenko 2005). Declines in freshwater invertebrate abundance and diversity in acidified rivers has been demonstrated across the literature, often due to declines in acid sensitive taxa (Feldman and Connor 1992; Courtney and Clements 1998). A secondary impact of acidification is the resulting increased solubility of metals in soils. These more readily wash out of soils into surface waters, increasing bioavailable concentrations of metals which impact invertebrate

communities (Campbell and Stokes 1985; Herrmann et al. 1993). In light of these implications and the transboundary nature of air pollution, international legislation has been put in place. For instance, the United Nations Convention on Long-Range Transboundary Air Pollution and the Gothenburg Protocol (Menz and Seip 2004; Grennfelt et al. 2019). This in hand with a trend for de-industrialisation within Europe has resulted in reduced emissions, acid deposition and the broad scale recovery of surface waters from acidification (Evans et al. 2001; Davies et al. 2005). Sulphur dioxide and nitrogen oxide emissions in Europe have been declining, with a respective decline of 97 % and 72 % within the UK in the years 1970-2017 (Vestreng *et al.* 2007; European Environment Agency 2018a; DEFRA and National Statistics 2019). Additional activities such as liming have been used to raise the pH of some rivers in North America and Europe, which has had varied effectiveness (Fjellheim and Raddum 2001; Clair and Hindar 2005; Mant et al. 2013). Kernan et al. (2010) found clear evidence of chemical and biological recovery in acidified lakes and streams in a 20-year study across 22 sites in the uplands of the UK. Despite the reductions in acid deposition, the ecological recovery within improving waters is patchy (Bradley and Ormerod 2002; Monteith et al. 2005; Ormerod and Durance 2009). This is cited to be because of a variety of factors, including repeated acid episodes, dispersal and reinvasion constraints and other changes in environmental conditions (e.g. climate) (Bradley and Ormerod 2002; Yan et al. 2003; Monteith et al. 2005; Layer et al. 2011).

Another source contributing to the acidification of rivers and other freshwater systems is acid mine drainage. Here material bearing sulphite is exposed to oxygen and water, leading to the release and dissolving of sulphate and soluble metal ions, producing sulphuric acid (Akcil and Koldas 2006). This can occur while mines are active and when these have been abandoned. As with acid deposition, toxicity of waters as a result of acid mine drainage is also related to the increased bioavailability of metals at lower pH, leading to the mobilisation of heavy metals from mine workings (which are often associated with the mining of heavy metals) (Campbell and Stokes 1985; Gilchrist *et al.* 2009). The impact of acid mine drainage on lotic systems is varied, impacted by the dilution and buffering capacity of receiving waters (Gray 1997). A review by Hogsden and Harding, (2012) analysed the food web impacts in acid mine drainage impacted rivers. Generally, this found impacted rivers to have shorter food webs, limited species diversity and number, with rivers largely dominated by a few tolerant species. Diversity and abundance of invertebrate populations within acid mine drainage impacted rivers is generally reduced (Koryak *et al.* 1972; Gray 1998; Winterbourn 1998).

### 2.8.1.5 Pesticides

Pesticides are used widely across the world within agricultural production systems, varying in their application, use (e.g. insecticides, herbicides, fungicides) and chemical properties. Their broad function, however, is that of protecting crops and enhancing yields (Aktar 2011). Pesticide application is not just a rural issue, with applications within urban areas, in domestic settings, public and amenity areas for pest and weed control (Kristoffersen et al. 2008; Meftaul et al. 2020). Within the UK, agriculture and horticulture are the predominant users of pesticides (Thomas 2001). In Great Britain, while the total weight of annual pesticide use has almost halved between the years 1990 and 2015, the area of land treated has more than doubled throughout this period (Goulson et al. 2018). Residues of pesticides have the potential to reach beyond the locations of their targeted application via diffuse sources (e.g. atmospheric, overland, subsurface) and point sources (e.g. spillages and consented discharges) (Carter 2000). Surface runoff and wastewater effluents (due to incomplete removal of pesticides) are considered among the most important entry pathways to waterways (Holvoet et al. 2007; Campo et al. 2013; Le et al. 2017). It has been shown that factors such as, weather condition, soil type, land use and pesticide properties influence the loss of these agrochemicals to watersheds, as with artificial fertiliser applications (Capel et al. 2001; Cui et al. 2020).

Agricultural pesticides have been described as one of the best ecotoxicologically categorised and regulated groups of contaminants (Beketov *et al.* 2013). Within the European Union the current standards require that a substance is only authorised if there are no unacceptable effects on the environment, although this is not always effective (European Parliament and Council 2009; Stehle and Schulz 2015b; Brühl and Zaller 2019). These include critique of the limited research into pesticide interactions with other stressors, pesticide mixtures and the limitations of the subsequent environmental risk assessment after a pesticide is in use. These criticisms are founded by the subsequent banning of many pesticides following research external to the environmental risk assessments required of regulations (Storck *et al.* 2017).

Pesticides vary in their toxicity and application methods, which can result in varied consequences to non-target organisms (Carter 2000; Sánchez-Fortún and Barahona 2005; Wang *et al.* 2012b; Cui *et al.* 2020). As a wide range of pesticides are used within agricultural settings, the resulting mixtures within aquatic systems can act synergistically and antagonistically (LeBlanc *et al.* 2012). Once within the freshwater system, processes such as biodegradation, photolysis and sorption can alter the impact of pesticides. These are dependent on the environmental conditions within the aquatic system and the properties on the chemical. In rivers, the sorption behaviour of pesticides strongly influences their final destination, due to the influence of this on transport and degradation of the pesticide (Holvoet *et al.* 2007). For example, pesticides which are highly persistent within the environment have the potential to accumulate, leading to higher and more toxic concentrations (Morrissey *et al.* 2015).

Pesticides in surface waters are considered among the major stressors of freshwater systems (Vörösmarty et al. 2010; Beketov et al. 2013; Stehle and Schulz 2015a). The roles of these chemicals on the degradation of global surface waters is thought to have been underestimated, in part due to a lack of comprehensive quantitative analysis and the rigorous testing and regulation of these chemicals (Beketov et al. 2013; Stehle and Schulz 2015a). River invertebrates have varying tolerances to pesticides and their concentrations within the water, resulting in wide ranging consequences for these invertebrate communities (Liess and Von Der Ohe 2005; Misaki et al. 2019). In a study using regional stream invertebrate taxa richness data from Germany, France and Australia, pesticides led to declines in species and family richness (Beketov et al. 2013). In France and Germany effects on biodiversity were detected at concentrations which are considered environmentally benign under legislation (Beketov et al. 2013). Pesticides effects on freshwater communities can also have implications for ecosystem function, such as leaf litter breakdown (Schäfer et al. 2012b; Schäfer et al. 2012a). It has been suggested that climate change will have implications on the use of pesticides, in addition to changes in their fate and behaviour within the environment (Bloomfield *et al.* 2006).

#### 2.8.1.6 Persistent Organic Pollutants

Persistent organic pollutants (POPs) due to their persistence, bioaccumulation potential and toxicity have been widely restricted in their use and production across the UK and

internationally (DEFRA, 2017; Stockholm Convention, 2017). Under the Stockholm Convention (United Nations Environment Programme, 2017), an international environmental treaty which entered into force in 2004, 26 POPS were restricted in their use. These include some pesticides such as aldarin and dichlorodiphenyltrichloroethane (DDT) which were widely used in agriculture and industrial chemicals such as polychlorinated biphenyls (PCBs) which had widespread uses in paints, polymers, adhesives and fire retardants (Borja et al. 2005). Despite the legal restrictions on the use of these chemicals, these legacy pollutants remain present and stable in the natural environment at low levels (Fujii et al. 2007; Rasmussen et al. 2015; Zhao et al. 2020). The persistent nature of POPs also leads to high potential for atmospheric and aqueous long-range transport from their original sources (Wania 2003; Nøst et al. 2018). These can also accumulate within bottom sediments, reducing concentrations within the water column and leading to high concentrations of POPs within suspended and bed sediments, which can subsequently be resuspended (Vane et al. 2010; Xia et al. 2013; Dong et al. 2016). Continued contributions to freshwaters include illicit use, runoff from landfill and sediment bound POPs from agricultural land/industrial sites (McKnight et al. 2015).

The widespread contamination of freshwaters with POPs is hazardous to organisms within and associated with the aquatic environment. The mechanisms behind this include endocrine disruption (Windsor *et al.* 2018) and neurotoxicity (Vasseur and Cossu-Leguille 2006; Mariussen 2012). POPs have been demonstrated influencing a variety of taxonomic groups (Harmon 2015), including microbes (López-Doval *et al.* 2010), invertebrates (Zou and Fingerman 1997; Bernatowicz and Pijanowska 2011), fish (Berg *et al.* 2016) and aquatic birds (Albanis *et al.* 1996; Lundholm 1997). The hydrophobic and hydrophilic nature of POPs results in the accumulation of these within freshwater organisms and magnification across the aquatic food web (Streets *et al.* 2006; Bizzotto *et al.* 2009; Verhaert *et al.* 2013). The bioaccumulation of aquatic organisms are influenced by different traits, such as life history (e.g. lifespan), morphological traits (e.g. mouthpart morphology), and trophic habit (e.g. feeding preferences) (Sidney *et al.* 2016; Windsor *et al.* 2019a). This makes for a relatively high risk from POPs despite relatively low environmental concentrations and reductions in environmental additions (Windsor *et al.* 2018). The legacy environmental implications of these pollutants within river sediments is of particular concern, with the neglect of legacy pollutants potentially resulting in the underestimation of sediment toxicity by up to 90 % (Rasmussen *et al.* 2015). Due to their use as pesticides along with their persistent and transient nature, POPs have the potential to be a vastly underestimated threat to rivers. Future implications of climate change on POPs within the environment have been reviewed by Wang *et al.* (2016), who suggested that climate change could increase the bioavailability of POPs while increasing rates of degradation.

#### 2.8.2 Modifications of rivers

#### 2.8.2.1 Physical modifications

Rivers within the UK are heavily modified systems, with modifications carried out over centuries and to varying degrees (Mainstone and Wheeldon 2016). For example, in England just 65 % of non-estuarine lotic surface waters are considered natural, with the remaining 7 % artificial and 28 % heavily modified (Environment Agency 2015a). The most heavily modified rivers tend to be concentrated in more urban areas and lowland fens, while the uplands are made up of the most natural rivers (Environment Agency 2010; Environment Agency 2015a). Modifications include re-sectioning of river lengths, for instance deepening or widening channels to reduce flooding of adjacent land. In England, Wales and the Isle of Man this accounts for almost 43 % of river lengths (Environment Agency 2010). Other modifications include in-channel structures, such as weirs and sluices. Bank stabilisation is another modification, used to prevent movement of the river channel and reinforcement of banks with solid structures e.g. concrete (with around 8 % of river length reinforced in some way) (Environment Agency 2010). The varied nature of these modifications means that the implications on river ecosystems differ.

The natural state of rivers allows for a complex, ever changing mosaic of patches, with differences in hydrology, geomorphology and resulting ecological characteristics (Frissell *et al.* 1986). Changes are driven by the natural processes within rivers, such as flow and sedimentation. Modifications often constrain these processes which drive river corridor form, dynamics and biocomplexity. This often leads to reduced connectivity with floodplains and riparian systems, which has largely been seen to have negative implications on biodiversity and ecological integrity within rivers (Gurnell *et al.* 2007). While these types of

modifications are considered to be a more characteristic problem in urban rivers, it is important to consider the potential ecological implications of physical modifications of rivers overall (Gurnell *et al.* 2007). Particularly as these lead to reduced complexity of river systems and have been found to have water quality and ecological consequences.

#### 2.8.2.2 Artificial changes to the flow regime

River flow rate can naturally vary seasonally and across catchments, however human activities have the potential to lead to deviations in the natural flow regime. These artificial changes in flow regime can be driven by changes to land uses, which can alter the interception of water before it reaches waterbodies or recharges groundwater (Robinson and Dupeyrat 2005; Zhao et al. 2012; Mishra et al. 2014). Other activities, such as groundwater extraction, diversion of water and damming also lead to direct alterations in the natural flow regimes of rivers. River regulation, through activities such as damming (for water provision and hydropower generation), can lead to stark changes in the physical and ecological characteristics of rivers upstream and downstream structures. Physical changes include more homogenous flows, due to losses in seasonal discharge patterns, whereas changes in water velocity can alter dissolved oxygen and sedimentation (Poff et al. 2007; Olden and Naiman 2010; Renöfält et al. 2010). Other ecological implications include, reduced energy flows and reduced migration due to fragmentation. These have been seen to alter river invertebrate diversity, community assemblages and abundance, both during construction and following the longer-term regulation driven changes in flow and water quality (De Jalon *et al.* 1994; Maynard and Lane 2012; Gillespie *et al.* 2015).

Another less specific example of artificial changes in the flow regime are reduced flows, driven by the human demand for water, for uses such as agricultural irrigation, industry, and domestic use. Higher water demand is generally aligned with times where flows are generally lower, which can exacerbate already lower flows and have a disproportionate effect on flow rates (Gasith and Resh 1999; Suren *et al.* 2003; McKay and King 2006). The consequences of anthropogenic low flow events on rivers varies, due to antecedent conditions (e.g. if seasonal low flow events are natural within a river), duration, timing and seasonality, magnitude, rate of change and frequency of low flow event (Rolls *et al.* 2012).

The impacts of artificial changes to flow regimes on the ecology of lotic systems range from more direct losses of habitat to the implications of lower flows on the water quality and the

consequences of this on biota. For example, physical losses in habitat can result in reductions of diversity where connectivity and habitat diversity is reduced (Dewson, James and Death, 2007). Additionally, the reduced flows can lead to declines in flow dependent taxa from invertebrate communities, which can influence the community composition (Dewson, James and Death, 2007; Brooks, Chessman and Haeusler, 2011). Water quality changes include decreased dissolved oxygen and increased temperature (Rolls *et al.* 2012). There is also the potential that low flows influence energy sources and flows within river systems, with low flows slowing the rate of energy transfer. For example, lower flows lead to reduced suspension and therefore retention of benthic organic matter, which can influence invertebrate communities (Boulton and Lake 1992; Rolls *et al.* 2012; Arroita *et al.* 2017).

Broad responses of invertebrates to low flow events vary, although low flow events largely lead to reductions in richness, abundance and diversity (Dewson, James and Death, 2007; Sabater *et al.* 2018). The responses to low flow events vary across stream types, in the UK Armitage and Petts (1992) found that water abstraction had less of an effect on species richness within upland streams compared to smaller lowland streams. Climate change has the potential to increase low flow events within some lotic systems, this combined with higher projected water demands by human populations has the potential to heighten already human intensified low flow events (Oki and Kanae 2006; Rolls *et al.* 2012).

#### 2.8.3 Invasive species

Invasive species are considered to be a major threat to global biodiversity (Simberloff *et al.* 2013), with freshwater ecosystems especially affected by non-native species (Sala *et al.* 2000; Leprieur *et al.* 2007). Intentional vectors of introduction include the stocking of lakes and rivers with species for aquaculture or sport (Gido and Brown 1999; Holdich *et al.* 2014) and the trade in ornamental plants and animals (Keller and Lodge 2007). Unintentional vectors include ballast water (Ricciardi 2006) and equipment from recreational anglers (Anderson *et al.* 2014; Smith *et al.* 2020). Although not all non-native species which arrive or establish outside of their natural range become invasive, this is the first step for a species to potentially become invasive (Kolar and Lodge 2001; Colautti and MacIsaac 2004; Strubbe *et al.* 2019). Britain has seen an increase in the number of known non-native freshwater species in the last two centuries, where a history of international trade and travel is thought to be responsible for a large number of these introductions (Keller *et al.* 2009; Jackson and

Grey 2013; Roy *et al.* 2014). The consequence of this has amounted to a huge economic cost (Cuthbert *et al.* 2021). Britain is currently committed to a variety of international, European and domestic policies aimed at reducing the impact on invasive non-native species (DEFRA 2003; The European Parliament And The Council Of The European Union 2014; Great Britain Non-native Species Secretariat 2015).

Freshwater systems are considered to have high susceptibility to invasions by non-native species (Moorhouse and Macdonald 2015). While the majority of known established non-native species in Britain are terrestrial, freshwater species are considered among the most likely to be invasive, due to higher dispersal abilities than terrestrial species (Gherardi *et al.* 2008). Rivers are particularly exposed to invasive species due to their connective nature, furthermore the high frequency of human access and disturbance of some river environments makes these especially vulnerable to invasion (Keller *et al.* 2011). More remote waterbodies, such as rural headwater streams, should be less exposed to invasion due to this remoteness and as colonisation largely occurs at a faster rate in a downstream direction (Bubb *et al.* 2004; Clarke *et al.* 2008). This makes invasive species a greater stressor of urban than rural areas, due to lower levels of access and disturbance.

It has been suggested that climate change will influence the instances of non-native arrivals, successful colonisations and the severity of invasions (Rahel and Olden 2008). Particularly within Europe where non-native species colonisations and populations have been otherwise restricted by lower temperatures (Hesselschwerdt and Wantzen 2018). Future scenarios of other river stressors will also impact the potential implications of invasive species in the UK, although there is conflicting evidence of environmental degradation leading to higher invasibility (Keller *et al.* 2011). Concern has also been raised of the potential implications of invasive species on the reliability of freshwater macroinvertebrate biomonitoring tools, due to the modification of native community structure (Macneil *et al.* 2013; Ercoli *et al.* 2015; Mathers *et al.* 2016b).

# 2.8.4 Climate change

It has been suggested that the impact of climate changes on rivers will be complicated (Palmer *et al.* 2009). Climate change presents an amalgamation of stressors varying across different scales, including temperature changes, elevated atmospheric CO<sub>2</sub> along with increased rate and intensity of drought/ extreme flow events (Collins *et al.* 2013; Lowe *et al.* 

2019). These factors combined with their chemical and ecological implications, will further amplify the impact of other stressors on river ecosystems (Jeppesen *et al.* 2010; Moss 2011; Charlton *et al.* 2018). It has been suggested that rivers are among the most sensitive ecosystems to climate change (Ormerod 2009; Woodward *et al.* 2010). Due to the wide-reaching impact of climate change across ecosystems, this will likely impact communities in rivers across the board. In a long-term data analysis of European stream invertebrate communities Jourdan *et al.* (2018) did, however, find that there was a stronger impact of temperature on sensitive taxa in agricultural regions. In terms of range shifts in response to rising temperatures, there is a potential that upland regions may be disproportionately impacted due to the inability of species to retreat to higher latitudes and altitudes (Hari *et al.* 2006; Heino *et al.* 2009; Tierno de Figueroa *et al.* 2010).

Studying the impact of climate change on rivers and the response of invertebrates is particularly challenging. This is due to limited long-term studies, varying trends in current climatic changes at different scales and contrasting projections in future climatic changes (Floury *et al.* 2013; Watts *et al.* 2015; Garner *et al.* 2017). Other large climatic variations add another level of complexity, for example the North Atlantic Oscillation (NAO) which can also influence temperature, precipitation and therefore lead to changes in in water quality (Blenckner and Hillebrand 2002; West *et al.* 2019). Despite this, the NAO can also act as a tool to illustrate the strength of response in river physiochemistry and ecology (e.g. growth, phenology, persistence of invertebrate communities) to smaller scale climatic variations. This can be used to indicate how rivers could respond to larger scale climate change (Ottersen *et al.* 2001; Blenckner and Hillebrand 2002; Briers *et al.* 2004).

#### 2.8.4.1 Temperature changes

In terms of the impact of temperature changes within rivers, the turbulence within these systems means that they are well mixed so consequently respond to changes in atmospheric temperatures readily (Kaushal *et al.* 2010). Warming trends in rivers have already been observed (Durance and Ormerod 2007; Durance and Ormerod 2009; Kaushal *et al.* 2010; Orr *et al.* 2015), although other anthropogenic activities are considered to potentially contribute somewhat. Including human induced changes in flow regimes, discharges of treated wastewater and urbanisation (Kaushal *et al.* 2010). Water temperature has been cited as one of the most important variables governing river habitats, due to its control on

biogeochemical processes, ecosystem dynamics and water quality (Caissie 2006; Hannah and Garner 2015).

In terms of biogeochemical processes, as most river organisms are ectotherms, changes in water temperature affect survival, distribution, growth and phenology (Hawkins et al. 1997; Daufresne et al. 2004). Shifts in species and communities of stream invertebrates have been observed, with taxa shifting to more suitable thermal conditions where temperatures have risen (Daufresne et al. 2004). The implications of temperature rises in rivers therefore have the potential to change assemblages of river invertebrates, due to differing thermal tolerances (Stewart et al. 2013). In lakes, changes in species composition have also been observed with rising average temperature (Burgmer *et al*. 2007). Growth rate of some invertebrates has been observed increasing under warmer conditions, in experimental manipulations and warmer years driven by the positive phase of the NAO (Hogg and Dudley Williams 1996; Briers et al. 2004). Temperature is known to play a role in phenology of river invertebrates, including on timing of emergences and generation time (Thackeray et al. 2010; Everall et al. 2014; Glazaczow et al. 2016). Temperature induced changes in the phenology, assemblages and growth of other organisms, such as fish and aquatic primary producers will generate further changes in assemblages and diversity of aquatic macroinvertebrates (Buisson et al. 2008; Thackeray et al. 2010; Shuter et al. 2012).

Higher temperatures increase the rate of biological and chemical processes, which drive these changes in physiochemical processes. Results of this include increased rates of decomposition (Battle and Mihuc 2000; Taylor and Chauvet 2014), primary production (Enquist *et al.* 2003; Acuña *et al.* 2008) and dissolved oxygen concentration (Cox and Whitehead 2009). These changes can further lead to changes in the ecology and water quality of rivers through other processes. Dissolved oxygen is also influenced by climate driven changes in flow rate, due to warmer conditions and reduced precipitation reducing oxygen saturation levels in rivers (Cox and Whitehead 2009). The effects of lower dissolved oxygen on river invertebrates include reduced grazing, survival and in mayflies movement to areas with higher velocity where exposure to predation is higher (Lowell and Culp 1999). It has also been observed to change assemblages of invertebrates and fish, due to varying tolerances of taxa to low dissolved oxygen concentrations (Justus *et al.* 2014).

## 2.8.4.2 Changes in flow regime

Climate change impacts on the hydrological conditions of rivers have been observed, with projections suggesting this is likely to be driven by changes in the rates of precipitation and evaporation (Bates *et al.* 2008). Projections and implications of these changes on river flows within the UK have been variable, although much research suggests the likelihood of increased winter flows and reduced summer flows (Sanderson *et al.* 2012; Watts and Anderson 2016; Garner *et al.* 2017). Flow regimes vary in rivers, in some regions these have consistently high or low flows while other are more variable (Poff *et al.* 1997). Tolerances of different invertebrates to high, low and variable flows vary, meaning changes in flow regime impact invertebrate diversity, density and composition (Bêche and Resh 2007; Datry *et al.* 2014; Lobera *et al.* 2017). Changes to flow regime can also influence other stressors of rivers, such as dissolved oxygen concentration (Cox and Whitehead 2009) and dilution of pollutants (Bloomfield *et al.* 2006; Rice and Westerho 2017).

At the more severe end of changes to flow regime are extreme events, such as droughts, flooding and heatwaves (Dai 2013; Fischer and Knutti 2015). Future extremes that are projected could contest the resilience of rivers and streams and the tolerances of biota to changing conditions and disturbance (Chadd *et al.* 2017). Where extreme events increase in frequency, intensity or change in timing this could result in changes of invertebrate assemblages. Likely driven by these extreme events impeding on the ability of invertebrates to persist under unfavourable conditions and recover or recolonise following perturbations (Boulton 2003; Chadd *et al.* 2017). Climatic extremes can lead to the passing of critical thresholds, the point or points where a relatively small change in conditions provokes a disproportionately large biotic response (Scheffers *et al.* 2016).

## 2.8.4.3 Interactions with other stressors

The impact of climate change on rivers extends beyond the implications of climatic changes on taxa, due to the potential for interactions with other freshwater stressors (Heino *et al.* 2009). For example, eutrophication, pollution and invasive species (Durance and Ormerod 2007; Rahel and Olden 2008; Feuchtmayr *et al.* 2009; Piggott *et al.* 2015). Drivers of these interactions are varied, for example the changing dilution or discharge of pollutants can occur due to changes in flow (Ormerod 2009). Therefore, the consequences of these interactions, while not necessarily all negative, have the potential to result in unexpected ecosystem responses (Woodward *et al.* 2010). The study of climate changes and multiple stressors which have occurred up to this date provides a challenge in itself, due to the range of changing natural and anthropogenic factors that river systems are already subjected to (e.g. discharge, light, water and land management practices). This combined with limited long-term studies and data also presents a challenge in demonstrating responses of freshwater ecosystems to anthropogenic climate changes (Vaughan and Ormerod 2014b; Garner *et al.* 2017).

## 2.8.5 Emerging threats

Emerging threats to freshwaters were outlined by Reid *et al.* (2019) which follows on from the seminal paper by Dudgeon *et al.* (2006) which defined the five leading causes of population declines and range reductions in global freshwaters (overexploitation, water pollution, flow modification, destruction/degradation of habitat, and exotic species invasion). This paper identifies 12 emerging threats to global freshwaters, although a variety of these are not necessarily applicable to UK rivers. Those which are relevant include changing climates, E-commerce and invasions, infectious diseases, harmful algal blooms, expanding hydropower, emerging contaminants, engineered nanomaterials, microplastic pollution, light, and noise, and cumulative stressors. Of the emerging risks identified, a variety of these have been discussed within the wider body of this literature review due to the higher degrees of understanding of the stressor and effects on freshwater systems. Those which have the most limited understanding of the potential severity of effects of biodiversity include emerging contaminants, engineered nanomaterials and microplastic pollution. Light and noise pollution is also discussed below as these are rarely discussed with reference to freshwater environments.

# 2.8.5.1 Microplastics

Microplastics, plastic particles less than 5 mm, have been cited as a potential major threat to aquatic ecosystems globally (Avio *et al.* 2017). Concentrations of microplastics have been reported across rivers in the UK (including in rural headwaters) (Hurley *et al.* 2018). These include microplastics intentionally produced (e.g. in cosmetics or exfoliating scrubs) and those formed from degradation of macroplastics (e.g. litter, washing of synthetic fibres). Sources of entry to rivers include from sewage treatment works, where microplastics not filtered out of effluent can be discharged into rivers or incorporated into sludge (Zubris and Richards 2005; Edo et al. 2020). Around 80 % of sewage sludge produced in the UK is applied to agricultural land, which through runoff following application has the potential to enter watercourses (DEFRA 2012). Runoff from septic tanks, has also been referred to as a potential source of microplastics to rivers (Horton et al. 2017). Other sources of entry include the degradation of macroplastics in the environment, from sources such as; litter, landfills, industrial agriculture (e.g. polytunnels) and roads (e.g. road marking paints). Once within the river, plastics can be transported to sea, deposited in sediments, adsorb other pollutants and change properties due to degradation (Turner and Holmes, 2015; Siegfried et al. 2017; Hurley, Woodward and Rothwell, 2018; Klein et al. 2018). Interaction of microplastics with freshwater biota has been recorded, with studies documenting ingestion of microplastics by organisms such as freshwater fish (Collard et al. 2018; Horton et al. 2018) and river invertebrates (Hurley et al. 2017; Windsor et al. 2019b). Concern has been raised surrounding negative impacts of this on aquatic biota, including on feeding, growth and reproductive behaviour. Research using freshwater aquatic organisms has, however, been limited (where much focus has been on marine biota) (Ma et al. 2019; Meng et al. 2020). Some studies have demonstrated no negative implications (Weber et al. 2018) while some have observed adverse impacts on growth and reproduction (Au et al. 2015; Redondo-Hasselerharm et al. 2018).

#### 2.8.5.2 *Emerging contaminants*

Pharmaceuticals and personal care products are emerging contaminants which are becoming an increasingly reported risk to freshwaters (aus der Beek *et al.* 2016; Ebele *et al.* 2017; Patel *et al.* 2019). Pharmaceuticals used in human and veterinary medicine are increasing in volume and number (Klein *et al.* 2018; Patel *et al.* 2019). Personal care products (PCPs) include products such as, hair colouring, toothpaste, and shampoos. Human pharmaceuticals and PCPs are largely excreted and emitted into the sewerage system after use, entering the aquatic environment via treated effluents from sewage treatment works (Comber *et al.* 2018). These can also enter the aquatic environment similarly to microplastics in the application of sewage sludge to agricultural land (Ivanová *et al.* 2018). Veterinary pharmaceuticals are widely released into the environment, including directly to land when used in pasture animals, within aquaculture, and indirectly when manure is applied to land (Boxall *et al.* 2003; Watanabe *et al.* 2010; Kim *et al.* 2016). These are increasingly being detected in aquatic environments in the UK (Kasprzyk-Hordern, Dinsdale and Guwy, 2008; White *et al.* 2019).

Various pharmaceuticals have been observed bioaccumulating within aquatic organisms, including fish and invertebrates (Brodin *et al.* 2014; Ruhí *et al.* 2016). Effects on aquatic biota include antibiotic resistant microorganisms, in addition to changes in growth, behaviour, reproduction and feminization in some aquatic species (Corcoran *et al.* 2010; Patel *et al.* 2019). Despite this, evidence of the effects of pharmaceuticals in aquatic invertebrates has been limited (Brooks *et al.* 2003; Huerta *et al.* 2012).

## 2.8.5.3 Nanomaterials

Nanomaterials are materials which contain nanoscale structures sized between 1 to 100 nm. The nanoparticles which make up these materials have especially high surface area to volume ratios, leading to physical and chemical properties which are often distinctive to conventional materials (Aitken *et al.* 2006). These currently have a variety of applications, in the medical field (Woodmansey and Roberts 2018), biosensing (Holzinger *et al.* 2014) and PCPs such as sunscreens (Schneider and Lim 2019). Many of these are understood to be entering freshwaters (Gottschalk *et al.* 2013; Dale *et al.* 2015). In rural rivers, a major potential source of nanomaterials are future agricultural applications, including pesticides, fertilisers and herbicides (Wang *et al.* 2016). Due to the limited information regarding the impact of nanomaterials in rivers, combined with knowledge of their toxicity and accumulation potential, nanomaterials are a potential emerging risk to rivers (Gottschalk *et al.* 2013).

## 2.8.5.4 Light and noise pollution

While light and noise pollution are highlighted as relatively well understood emerging stressors by Reid *et al.* (2019), in terms of noise pollution, much of the research has been conducted in marine environments (Kunc *et al.* 2016; Solan *et al.* 2016; Mickle and Higgs 2018). Noise pollution is observed at greater levels in more urban freshwater environments, source include; industry, shipping, traffic noise and recreational boating (Hildebrand 2009; Rountree *et al.* 2020). Broad implications of noise pollution on aquatic species include negative effects on development, physiology and behaviour of invertebrates and

vertebrates (Kunc *et al.* 2016). This could lead to direct impacts on invertebrates and indirect impacts due to altered predator responses (Holt and Johnston 2015).

Light pollution will be an issue of more urban environments. Implications of light pollution on river invertebrates include disrupting aquatic insect dispersal due to attraction to artificial lights which could reduce population sizes by direct losses of individuals, reproductive failures, or changes in sex ratios (Eisenbeis 2006; Perkin *et al.* 2014). Other implications include changes in predator and prey populations and foraging efficiency driven by artificial light, which will likely lead to changes in food webs (Perkin *et al.* 2011; Brüning *et al.* 2018).

## 2.8.6 Multiple stressors and interactions

As discussed, various stressors have direct implications on the ecology of river ecosystems, due to stressor driven changes in habitat, water quality and river organisms. Due to the nature of rivers connectivity to the surrounding terrestrial landscape and other lotic systems within a catchment, rivers are often influenced simultaneously by multiple stressors. In a study investigating the exposure of rivers in Europe to four main groups of pressures (hydrology, morphology, water quality, and connectivity) it was found that of the 9330 sites, rivers are largely impacted by more than one pressure (47 %). Lowlands were found to be especially impacted, with 90 % affected by a combination of all four groups (Schinegger *et al.* 2012). As a result, and due to the often unpredictable consequences of multiple stressors, there has been an increasing focus on the need for further study in this area (Townsend *et al.* 2008; Ormerod *et al.* 2010; Hering *et al.* 2015; Côté *et al.* 2016).

Multiple stressors within an ecosystem can have no specific interactions but act additively and multiplicatively, where the total effect of all stressors is equal to the sum or product of all stressors (Folt *et al.* 1999). Stressors can also have synergistic or antagonistic effects, which can result in the combined effects being larger or smaller than the individual stressor effects (Côté *et al.* 2016). Studies investigating multiple stressors have used variety of approaches and scales, including laboratory studies, field studies, mesocosm and big data analysis (Piggott *et al.* 2012; Schinegger *et al.* 2012; Wagenhoff *et al.* 2013; Jackson *et al.* 2016; Lemm and Feld 2017; Bray *et al.* 2019; de Vries *et al.* 2019). In a meta-analysis of multiple stressors in freshwater ecosystems, Jackson *et al.* (2016) investigated ecosystem responses to paired stressors. This found the net effects of stressors to be more frequently antagonistic than synergistic, additive, or reversed. This suggests that there could be a potential for co-adaptation within freshwater ecosystems, potentially minimising the net effects of multiple stressors. Alternatively, this could be attributed to the disproportionate effects of individual stressors on freshwater systems. It has been suggested that antagonistic effects could result in the underestimation of some stressors and their effects within rivers. For example, pesticides in rural river, which due to their interactions with other stressors such as fine sediments, through sorptive processes, could potentially conceal their true effects and concentrations (Bray *et al.* 2019).

A range of studies have demonstrated stressor interactions and effects on invertebrate communities, in predominantly mesocosm experiments. For example, Piggott *et al.* (2015) found that benthic invertebrate taxon richness and EPT (Ephemeroptera, Plecoptera and Trichoptera) richness decreases with fine sediment additions to a greater degree when temperature is also increased. Reductions of total invertebrate abundance with fine sediment additions have also been found to be amplified with phosphorous enrichment in some instances (Davis *et al.* 2018). Reduced flow rate has been also shown to increase the negative effects of sediment addition on invertebrate density and taxonomic richness (Matthaei *et al.* 2010) and EPT richness (Blöcher *et al.* 2020).

# 2.9 Recovery of rivers following improvements

Throughout this literature review some cases of recovery within rivers has been discussed. Within the UK, Whelan *et al.* (2022) explored how water quality has been changing since the industrial revolution. This paints a mixed picture with substantial reductions of some pollutants while others have increased and are likely to continue to increase. Some improvements in water quality would be expected to be reflected in ecological recovery, which in some cases have been observed (Vaughan and Ormerod, 2012; Outhwaite *et al.* 2020). However, following management and restoration measures in streams and their catchments, evidence of recovery is often limited (Worrall *et al.* 2009; Meals *et al.* 2010; Palmer *et al.* 2010; Feld *et al.* 2011; Friberg *et al.* 2014; Verdonschot *et al.* 2016; Lorenz *et al.* 2018). This has partly been attributed to limitations in the monitoring and experimental design of some studies and the effects of multiple stressors, which may complicate ecological responses (Hamilton 2012; Friberg *et al.* 2016). Time lags in the responses to restoration can also delay any measured recovery. This can be reflected in the time for

restoration actions to garner a desired effect, such as time for changed land management practices to influence nutrient additions to rivers, and then time for biota to respond to these changing conditions (Meals *et al.* 2010). Several of these can be attributed to hydrological and biogeochemical processes, which can result in long timescales following reduced stress on a river system to be evident within the river and on its biota. In addition, legacy stressors can take longer before benefits following improvements are observed. For example, the time lag between land nitrate application to reach rivers through groundwaters has resulted in continued elevated nitrate concentrations in UK rivers despite mitigation efforts dating back decades (Vero *et al.* 2018). Regime shifts and alternative feedback regimes within ecosystems can also limit recovery within some systems following interventions, where new stable states have been reached making recovery to the prior conditions irreversible (Scheffer and Carpenter 2003; Jarvie *et al.* 2004). This is highlighted by Jarvie *et al.* (2013) as a reason for mitigations in stressors (e.g. phosphorus additions) to not necessarily result in linear improvements in water quality or ecology within short timeframes and without other interventions.

## 2.10 River invertebrate trends in the UK

The availability of research investigating long-term trends across large regions is relatively limited across the UK and further afield (Haase *et al.* 2023). Many long-term analyses of river invertebrate trends focus on trends within particular systems or regions, often with aims to answer questions regarding particular stressors or management implications (Gibbins *et al.* 2001; Bradley and Ormerod 2002; Parr and Mason 2003; Durance and Ormerod 2009; Langford *et al.* 2009). Research across England and Wales has investigated how invertebrate communities have changed across England and Wales in the years 1991 – 2008 (Vaughan and Ormerod, 2012). This also considered how change varies across gradients of urban/rural land use and altitude. Richness at the family-level of river invertebrates in England and Wales were found to have increased by almost 20 %. This showed a shift towards taxa of more well-oxygenated conditions, higher water quality and faster flows, such as taxa within EPT families. Improvements were found to be greater in more heavily urbanised catchments, with these gaining taxa at twice the rate of rural catchments. These changes over time were mostly attributed to improvements in water quality and changes in discharge. A deterioration was however found in the uplands of

northern England and mid Wales, where it is suggested that implications of changes in water chemistry and climate within these areas over the data period analysed.

Vaughan and Ormerod (2014a) investigated changes in prevalence of invertebrate families across England and Wales, in addition to spatial distribution of individual taxa over 21 years (1991-2011). This allows for the identification of taxa driving changes in richness and any taxa which may be declining and disguised by these overall trends. Mean taxon richness was found to increase over this time period, although there was a partial turnover of taxa. 76 % of families showed a significant change in overall prevalence, with Ephemeroptera and Trichoptera driving the majority of these increases. Upland taxa largely expanded across the lowlands, and in terms of changes in richness there were large increases in richness across northern and central England and south Wales. Whereas there was a small decline in richness in western Wales and southern England. These changes are largely attributed to positive changes in changes in water chemistry, supporting similar finding by (Vaughan and Ormerod, 2012).

Long-term trends in river invertebrate abundance within England were investigated by Powell *et al.* (2023), over the years 2002-2019. This study found that change through time varied across taxonomic and trophic groups. Carnivores and herbivores were found to increase in abundance, while decomposers declined. Trends varied for rivers of different typologies, with abundance in organic lowland rivers increasing at higher rates across taxa and trophic groups at large, while this declined the most in siliceous lowland rivers. The study adds further details, revealing potential consequences for ecosystem functioning as change through time was found to vary between trophic groups. This also updates analysis by Vaughan and Ormerod (2014a), similarly finding increases in Plecoptera while Ephemeroptera decreased (contrasting the increases observed from 1991 – 2011).

Outhwaite *et al.* (2020) investigated annual occupancy of terrestrial and freshwater species in the UK over the years 1970-2015, to quantify trends in average occupancy and differences between rare and common species over this period. The research found a sharp decline in freshwater invertebrate species from 1970-1992 and a rapid increase after this time. In terms of EPT taxa and aquatic bugs specifically, these are observed to have followed this decline until the early to mid-1990s and increase after this point. Largely overall occupancy in freshwater species is seen to have reached overall stasis since 2005. This study

comments on the timing of the lowest mean occupancy following the introduction of the European Urban Waste Water Treatment Directive in 1991 and regulatory changes in the water industry around this time (Saal and Parker 2000). While these increases in average occupancy have been observed, it is also noted that these must be put into the context of larger declines since the industrial revolution, and thus trends from the 1970s onwards should be considered as such. This study is consistent with other research which has indicated large-scale ecological recovery of English and Welsh rivers since 1990 in addition to other studies in Europe (Langford *et al.* 2009; Vaughan and Ormerod, 2012; Haase *et al.* 2019).

# 2.11 Conclusion- Background and overall project aims

Freshwaters and rivers are biodiversity hotspots (Lehner and Döll 2004; Balian *et al.* 2008; Strayer and Dudgeon 2010). Despite this, they have rates of biodiversity decline far greater than terrestrial and other aquatic systems (Jenkins 2003; Collen *et al.* 2014; WWF 2016; Sánchez-Bayo and Wyckhuys 2019). Rivers are highly associated with human activities both within the river and their surrounding catchments (Dudgeon *et al.* 2006; Vörösmarty *et al.* 2010). They are therefore subject to a whole host of pressure which are relevant to the UK and a wider context, as highlighted throughout this literature review. Throughout time the combination and dominance of these pressures have changed; driven by policy, land use, scientific understanding, and the emergence of new pressures (Table 2.4).

It is important that the ecological consequences of these stressors are well understood to inform policy and management decisions. Long-term studies are particularly important and can be used to unpick natural variations, as well as biotic and abiotic interactions from human driven stressors (Jackson and Füreder 2006; Burt *et al.* 2010; Lindenmayer *et al.* 2012). Due to the relative ease of surveying river invertebrates and ecological applicability, there is an abundance of macroinvertebrate data collected in the UK and globally (Bonada *et al.* 2006; Vaughan and Ormerod, 2012; van Klink *et al.* 2020). The availability of long-term data is well-suited for answering questions about the ecological status of rivers over time and the implications of stressors."

As outlined throughout this literature review, there is extensive research on the variables influencing river invertebrates (e.g. fine sediment, acidification, land use), often at the site

scale. Additionally, there is existing research investigating how invertebrate communities have changed over time in the UK (Vaughan and Ormerod, 2012; Outhwaite *et al.* 2020; Powell *et al.* 2022) and across different spatial contexts, such as altitudes and catchment land use types (e.g. Vaughan and Ormerod, 2012; Powell *et al.* 2022). What is lacking, however, is a detailed national analysis that integrates these approaches: investigating how invertebrate communities change over time, the relationship between land use, water quality variables and invertebrate communities, and how relationships between these variables vary across England and Wales. This research aims to fill this research gap, providing a comprehensive national overview while also examining how and why these trends vary spatially, utilising a combination of detailed analyses.

This current project will aim to build on past research which has investigated UK wide national trends in river invertebrates and attempt to unpick the driving forces behind these changes (Vaughan and Ormerod, 2012; Vaughan and Ormerod, 2014a; Outhwaite *et al.* 2020). It will utilise national scale invertebrate data collected in the years 1991 - 2019 by the Environment Agency and Natural Resources Wales, building on previous analysis of this dataset (Vaughan and Ormerod, 2012; Vaughan and Ormerod, 2014). This national scale data will be used to quantify how river invertebrate communities have changed across England and Wales, and how this varies with factors such as land cover and altitude. A key challenge of this will be determining what variables are driving trends through time. Potential key drivers of changes (e.g. climate, nutrient concentration, fine sediment, land cover), discussed throughout this literature review, will then be tested to see how and if they have contributed to any ecological changes. This will utilise resources such as environmental monitoring data, land use and hydrological data from this time period. This initial big data analysis will be used to inform a smaller scale study, which will aim to gain further insight into the mechanisms driving these national changes.

# Chapter 3: Evidence of biological recovery in English and Welsh rivers over three decades

# 3.1 Summary

There is ongoing debate around the changing ecological status of European rivers, and in particular the challenge of managing the effects of multiple stressors under a changing climate. In the UK, there is intense public interest in both rural and urban rivers which prompts the need for contemporary evidence on trends and status. While recent studies have addressed water quality patterns, there has been little recent assessment of national biological trends. This chapter investigates changes in English and Welsh river macroinvertebrate communities over almost 30 years (1991-2019) using a network of nearly 4,000 survey locations. The aims of this chapter are to i) outline trends in taxonomic and functional richness, and community composition, ii) describe gains, losses and turnover of taxa, and the overall homogeneity of macroinvertebrate communities at a national scale, and iii) and explore how temporal trends varied with catchment characteristics. To ensure that outlined richness and compositional changes illustrated are representative of rivers across England and Wales, the selected sites are compared to a stratified-random sample of rivers (River Habitat Survey). Macroinvertebrate sampling locations were found to underrepresent smaller, steeper headwaters, and tended to be concentrated at slightly lower altitudes and higher catchment urbanisation. Therefore, a post-stratification procedure weights sites using the River Habitat Survey, to ensure that change through time is representative of English and Welsh rivers as a whole.

Taxonomic richness increased, primarily in the 1990s, whilst there was a shift towards communities typical of better water quality and well oxygenated conditions that continued throughout the study period. Improvements occurred in both urbanised and agricultural catchments, but with larger gains in urban areas, gradually homogenising macroinvertebrate communities as urban rivers closed the gap on rural ones. Overall, these results indicate continuing biological recovery, consistent with national scale trends in water quality. Results reemphasise the importance of looking at multiple facets of diversity, with periods of near-constant richness disguising changes in composition. Whilst the national-scale picture was broadly positive, as this chapter revealed how change through time varied across different

catchment variables, it would be essential to investigate how this varies across space (as land use differs across England and Wales).

## 3.2 Introduction

Freshwaters support disproportionately high biodiversity relative to their global surface area, but are at risk from multiple stressors ranging over pollution, morphological alteration, flow modification, invasive non-native species and human overexploitation (Balian *et al.* 2008; Strayer and Dudgeon 2010). In many cases, these pressures on freshwater ecosystems are a reflection of the land use, management practices and other activities carried out within their catchments (Allan *et al.* 1997; Allan 2004; Fierro *et al.* 2017; Nelson Mwaijengo *et al.* 2019). Activities such as water abstraction, flood defence, flow regulation and impoundment also have direct impacts (Ward and Tockner 2001; Lowe *et al.* 2019). These stressors are further exacerbated and complicated by climate change (Jeppesen *et al.* 2010; Moss 2011; Charlton *et al.* 2018), as thermal regimes and discharge patterns change (Palmer *et al.* 2009; Collins *et al.* 2013; Lowe *et al.* 2019). Taken together, these pressures have been implicated in steep declines in freshwater biodiversity, such as an estimated 84 % decline in global freshwater vertebrate abundance since 1970 (WWF 2020).

In contrast to this global picture of consistent decline, recent studies of freshwater invertebrate biodiversity reveal a more complex situation. Some studies have shown large declines in invertebrate abundance and diversity (Hallmann *et al.* 2017; Lister and Garcia 2018; Sánchez-Bayo and Wyckhuys 2019; Wagner 2020), whilst others have revealed substantial increases in recent decades (Crossley *et al.* 2020; Filippakopoulos and Knapp 2020; van Klink *et al.* 2020) or mixed responses through time (e.g. Baranov *et al.* 2020). Given the important role that invertebrates play in riverine food webs and ecosystem functioning (Morse 1971; Ollerton *et al.* 2011; Yang and Gratton 2014), coupled with their efficacy as indicators of environmental quality (Hodkinson and Jackson 2005; Bonada *et al.* 2006; Friberg *et al.* 2011), revealing these trends is a priority.

In the UK, the condition of freshwaters has become a topic of intense public interest in recent years and there is concern that river quality may be declining (RSPB, 2021; The Rivers Trust, 2021; House of Commons Environmental Audit Committee, 2022). Concerns have increased around ongoing point source pollution, particularly related to combined sewage

overflows and some types of intensive agriculture, coupled with threats from emerging pollutants such as microplastics, climate change and diffuse agricultural pollution (Surfers Against Sewage 2021; Water UK 2021). Recent assessment of national water quality reveal a mixed picture with some long-term improvements, for example with sanitary waste, but also areas of ongoing concern (Whelan *et al.* 2022). Importantly, alongside these water quality assessments, the biological quality of UK rivers has been monitored extensively by statutory agencies such as the Environment Agency and Natural Resources Wales, encompassing macroinvertebrates, fish and other taxa, underpinning both routine quality assessments and forming a valuable resource for studying long-term trends in river condition and the potential influences of water quality and land use (Durance and Ormerod, 2007, 2009; Ormerod and Durance, 2009; Dunbar, Warren, *et al.* 2010; Vaughan and Ormerod, 2012; Vaughan and Ormerod, 2014c; Mathers *et al.* 2019; Vaughan and Gotelli, 2019; Outhwaite *et al.* 2020).

Past analyses of biological data from UK rivers have revealed some substantial but nuanced recovery of macroinvertebrate communities from historical water quality problems. Vaughan and Ormerod (2012) estimated a near 20 % increase in richness in the years 1991-2008 and a shift towards taxa associated with better water quality, although trends varied across England and Wales, with the largest improvements in more urban locations. Outhwaite *et al.* (2020), using data from different monitoring schemes, found a similar increase in the prevalence of many freshwater macroinvertebrates after 1990 in Great Britain, which plateaued early in the 2000s: this translated into a similar temporal pattern in functional diversity (Greenop *et al.* 2021). Whilst water quality in urban areas has widely improved in the last 50 years due to changes in legislation, technologies, improved infrastructure and industrial decline (Langford *et al.* 2009), 71 % of UK land area is agricultural, with practices intensifying since the Second World War (Angus *et al.* 2009; DEFRA 2016). As a result, agriculture and rural land management contribute to the largest number of poor water quality assessments (Environment Agency 2019).

Several factors combine to make an updated analysis of national scale river invertebrate data important. First, over the last decade, attention to the changing status of the UK's rivers has grown, with some indicators revealing potential issues with combined sewer overflows and intensifying agriculture (Laville 2020; Cockburn 2021; Farhoud 2021; Stallard 2022).

Alongside this, concerns around emerging contaminants, climate change and cumulative stressors have increased as new data have become available (Reid *et al.* 2019). Second, recent analysis of water quality data has revealed contrasting trends for different pollutants (Whelan *et al.* 2022), against which the ecological benefits of past water quality improvements may have been exhausted (Vaughan and Gotelli 2019). Previous studies suggested a slowing in recovery around the late 1990s or early 2000s (Vaughan and Ormerod, 2012; Outhwaite *et al.* 2020), so there is a need to establish whether this has continued, or was simply a pause in recovery or prelude to decline. Finally, recent research points to subtle declines in functional diversity (e.g. Larsen *et al.* 2018) which may be overlooked by assessments of taxon richness alone.

Using a network of nearly 4,000 survey locations which were sampled over 29 years (1991-2019 inclusive), this chapter aimed to investigate trends in the river macroinvertebrate communities of England and Wales over 29 years (1991-2019 inclusive). Investigations sought to characterise biodiversity changes in depth, including functional and beta-diversity, and analysis of long-term change in relation to stream type and catchment land cover. Specifically, aims were to i) outline trends in taxonomic and functional richness, and community composition, ii) describe gains, losses and turnover of taxa, and the overall homogeneity of macroinvertebrate communities at a national scale, and iii) and explore how temporal trends varied with catchment characteristics. The work extends the timeframe of previous research (Vaughan and Ormerod, 2012; Outhwaite *et al.* 2020) by around 10 years to update national trend estimates. Methodology used by Vaughan and Ormerod (2012) was also improved, by reducing biases in the data to make the analysis more representative of English and Welsh rivers as a whole – in particular smaller waterbodies, which are often overlooked (Seager *et al.* 2012; Riley *et al.* 2018). It was predicted that:

- Hypothesis 1: Taxonomic and functional richness, and the prevalence of taxa associated with better water quality, will have remained stable over the last 10-15 years, continuing the pattern of the slowing or cessation of change in the late 1990s/early 2000s. This will be reflected in a decline and then stabilisation in inter-annual variation in invertebrate communities, leading to more consistent community composition through time.
- Hypothesis 2: Increases in richness and the prevalence of sensitive taxa will be greater and continue for longer within more heavily urbanised catchments. This in turn will result in

rivers becoming more homogenous at a national scale through time, as the communities in recovering urban streams converge on those in historically less polluted rural areas.

# 3.3 Methods

# 3.3.1 Macroinvertebrate data

Macroinvertebrate data collected over 29 years (1991–2019 inclusive) by two British environmental regulators, the Environment Agency and Natural Resources Wales, were collated for English and Welsh rivers. The data were filtered to include only samples collected in spring (March-May inclusive) using a standardised, 3-minute kick-sampling protocol, and subsequently sorted and identified in the laboratory. A joint quality-assurance scheme for England and Wales (1991–2009) indicated that the error rate was near-constant (Murray-Bligh and Griffiths 2022), and similar protocols remained in place subsequently for sample processing in the two countries. Sampling sites were included if they: i) had been sampled in at least four years during 1991-2019, including at least one sample in each decade (1991-2000, 2001-2010 and 2011-2019); ii) were not directly downstream from an effluent outfall; iii) included data on altitude, distance from source and channel slope; and iv) could have their catchments delineated from a digital terrain model. Criterion (i) represented a trade-off between maximising the number of sampling sites and temporal coverage within sites, whilst minimising site turnover in the data set. Catchments were delineated from a 50-m resolution digital terrain model (OS Terrain 50, Ordnance Survey) using ArcHydro tools (ESRI ArcGIS 10.7.1), apart from a small number of instances (e.g. where the drainage network had been modified and included loops). This filtering process left a total of 47,009 samples from 3982 sites, representing a mean of 11.8 years sampled per site (Figure 3.1). Although the overall national sampling effort declined between 1991 and 2019, there was little change in sampling effort at the 3982 sites over time (Appendix B Figure B.1).

Data were reduced to 78 composite and family groups to eliminate any effects of changes in taxonomy or the varying taxonomic resolution to which samples were identified to ensure consistency through time (see Vaughan and Ormerod 2014; Appendix B Figure B.1). Similarly, most analyses used presence-absence rather than abundance data due to variation

in how abundance was recorded and for consistency with previous studies. Taxon abundances were recorded as either raw counts or placed in log<sub>10</sub> abundance classes (e.g. 1-9 and 10-99 individuals). To use abundance, all data were harmonised into log<sub>10</sub> classes and converted to the midpoint for each class (e.g. the abundance for taxa in the 1–9 class was recorded as 5).



Figure 3.1 Locations of macroinvertebrate sites included in the calculation of temporal assemblage trends (n = 3,982 sites). Map contains OS data © Crown copyright and database right (2021).

# Macroinvertebrate metrics

Three metrics were used to summarise the macroinvertebrate community: i) family-level richness, ii) the first axis from a correspondence analysis (CA1) to summarise the major variation in community composition, and iii) functional richness to capture the diversity of traits, indicating the volume of niche space occupied by the community (Mason *et al.* 2005). Correspondence analysis was run with R's vegan package (Oksanen *et al.* 2019), repeating the analysis for presence-absence and abundance data, producing CA1<sub>PA</sub> (eigenvalue = 0.260) and CA1<sub>Ab</sub> (eigenvalue = 0.496) scores respectively. This, and all other analyses in this paper, were carried out in R v4.0.1 and RStudio v 1.2.5033 (R Core Team 2021; RStudio Team, 2020)

Functional richness used seven traits described by Tachet et al. (2010) that focused on food and habitat preferences: maximum size, feeding habit, food, locomotion and relation to substrate, preferred current velocity and preferred substratum (Appendix B Table B.2). Genus-level data on traits were downloaded from freshwaterecology.info (Schmidt-Kloiber and Hering 2015) in the form of affinities (weights) for the subcategories within each of the seven traits. These were converted to family-level traits by calculating each family's mean affinity for each subcategory from its constituent genera, weighting each genus by its relative abundance across the 2505 samples that formed the original RIVPACS calibration data for the UK (Wright et al. 2000; Monaghan and Soares 2012). Affinities were then rescaled so that the sum of the subcategories within each trait summed to one for each family (Vaughan and Ormerod, 2014). This was possible for 73 of the families which formed this part of the analysis (Appendix B Table B.1). Functional richness was calculated using the dbFD function in the R package FD (Laliberté *et al.* 2015), selecting four principal coordinate analysis (PCoA) axes using the methodology of Maire et al. (2015). A few samples (0.7 %) had to be removed where the number of taxa present was less than the number of PCoA axes.

#### 3.3.2 Trends in invertebrate assemblages

Temporal trends in macroinvertebrate richness, CA1 scores and functional richness were estimated for the time period 1991–2019 using generalised additive models (GAMs), fitted using R's mgcv package (Wood 2011). The approach followed Vaughan and Ormerod (2012, 2014), using Fewster *et al.*'s (2000) methodology for estimating smoothed trends after first weighting individual sampling sites to minimise bias in the sample site locations. This step was required because macroinvertebrate sampling locations under represented smaller, steeper headwaters, and tended to be concentrated at slightly lower altitudes and with higher catchment urbanisation (Appendix B Figure B.1) when compared to a stratified-random sample of nearly 5,000 English and Welsh rivers (2007–2008 River Habitat Survey (RHS) Baseline; Seager *et al.* 2012).

Modelling of temporal trends comprised three stages. Firstly, the probability of a river reach being sampled for macroinvertebrates was modelled as a function of a range of environmental variables. Secondly, sites had post-stratification weights calculated according to their probabilities of being sampled and thirdly, GAMs were fitted to model long-term
invertebrate trends using the post-stratification weightings. This process reduced biases by, for example, weighting macroinvertebrate sites in relatively under-sampled headwater streams more heavily in the GAMs. In the first stage, the 3982 invertebrate sites were combined with 4104 RHS Baseline sites located across England and Wales, and a binomial GAM was used to predict the probability of a river reach being sampled for macroinvertebrates based on 12 environmental variables that correlate with variation in invertebrate communities (Dunbar et al. 2010a; Dunbar et al. 2010b; Leathwick et al. 2011; Clapcott et al. 2012; Booker et al. 2015): i–iii) the percentage of urban or suburban landcover (pooled in this analysis as urban), improved grassland or arable agriculture calculated from the 1 km resolution UK Land Cover Map 2007 (Morton et al. 2014); iv) the proportion of each catchment underlain by calcareous geology from 1:625,000 scale maps (British Geological Survey/ Natural Environment Research Council); v) mean annual precipitation in each catchment - calculated from 1961–1990 averages mapped at 5 km resolution (UK Meteorological Office, Exeter, UK; (Perry and Hollis 2005); vi–vii) specific stream power (W m<sup>-2</sup>) and the base flow index (BFI) estimated following (Vaughan et al. 2013); viii) catchment area; ix-x) elevation and channel slope at the sampling location; and xi–xii) the easting and northing of the sampling location. Base flow index is the proportion of the river runoff derived from stored sources, and indicates how the discharge of a river may be influenced in periods of drought (Bloomfield et al. 2009; Rothwell et al. 2010b; Stoelzle et al. 2014). Stream power has been shown to be a good predictor of the physical habitat within the channel, including the predominant substratum (Vaughan et al. 2013). In the GAM, all variables were modelled as cubic-regression splines, with easting and northing modelled as a tensor product smooth, with the smoothness selected by generalised cross validation (Wood, 2011). Predicted probabilities of macroinvertebrate sampling were generated for the 8086 macroinvertebrate and RHS sites.

In the second stage, predicted probabilities of macroinvertebrate sampling acted as propensity scores for sites which were used to calculate post-stratification weights for each site (Rosenbaum and Rubin 1983). The RHS Baseline sites were divided into five adjustment cells based upon the quintiles of predicted probabilities. From that classification, the proportion of England and Wales's river network length within each of the five adjustment cells was calculated by: i) measuring the river length within the strata used for RHS sampling

(rivers shown on the 1:250,000 scale network and additional headwaters represented on 1:50,000 scale maps, within each 10 km national grid square; Seager *et al.* 2012); ii) dividing this by the number of survey sites in each stratum as a measure of river length represented by each survey site; iii) summing the length for all sites within each adjustment cell; and iv) dividing by the national total to express this as a proportion. Post-stratification weights, *w*, were then calculated for the macroinvertebrate sites that fell into each of the five adjustment cells, as  $w_h = rP_h/r_h$  where *r* was the total number of invertebrate sites (3982),  $P_h$  was the proportion of river length in adjustment cell *h* and  $r_h$  was the number of invertebrate sites in adjustment cell *h* (Little, 1993).

In the third stage, CA1 scores, and taxonomic and functional richness, were modelled as a function of year, using cubic regression splines with sites weighted using the poststratification weights (Vaughan and Ormerod, 2012). The models included the 12 site and catchment level environmental covariates to account for variation in macroinvertebrate assemblages between sites (Fewster *et al.* 2000; Vaughan and Ormerod, 2012). The degrees of freedom (smoothness) of the year term was fixed at nine, approximately 1/3 of the number of years, as this is a good compromise between identifying long-term trends and shorter-term (multiple-year) changes (Fewster *et al.* 2000). Bootstrapping, including the process of calculating post-stratification weights, was used to produce nonparametric 95 % confidence limits of the trends, based on 399 bootstraps. Significant positive and negative 'change points' (inflections) in the trend were identified using bootstraps of the trends, following (Fewster *et al.* 2000).

Annual, unsmoothed estimates of taxonomic and functional richness and CA1 scores were also calculated, using identical methods to the smoothed estimates, except that year was treated as a categorical variable.

As recommended by Hewson and Noble (2009), overall changes are quoted between the second and penultimate years of the time series (i.e. for 1992 v. 2018, rather than 1991 v. 2019), due to the lower reliability of smoothed estimates at the ends of time series.

#### 3.3.3 Changes in beta-diversity

A permutation approach was used to test for homogenisation of macroinvertebrate communities over time i.e. increasingly similar macroinvertebrate communities across locations. To control for variation in sampling frequency among sites, one sample was drawn at random from each site during each decade (1991-2000, 2001-2010 and 2011-2019 i.e. *n* = 3982 in each time window). The mean Jaccard dissimilarity was calculated among all pairs of sampling sites within each decade, and the process repeated 10,000 times, from which 95 % non-parametric confidence limits were calculated around the mean dissimilarity for each time window based on the 2.5 and 97.5 percentiles (Buckland 1984).

To assess the rate of macroinvertebrate community change through time further, the mean annual turnover across English and Welsh sites was calculated using the codyn package's turnover function (Hallett *et al.* 2016). Turnover between a pair of neighbouring years at a site is defined as:

 $Turnover = \frac{Families gained + Families lost}{Total families observed in both years}$ 

from which the national average was calculated. This was further split into its two constituents: the proportion of families gained (appearances) and lost (disappearances; Hallett *et al.* 2016). Sampling effects, accentuated by the presence of rare taxa, means that there will always be some apparent turnover between samples; hence, the focus of this analysis was to look for changes in the rates of turnover, gains and losses through time, rather than their absolute values. To test for trends, the proportion of turnover, gains or losses was regressed onto year using the lm function (R Core Team, 2021).

# 3.3.4 Community change versus catchment characteristics

A second set of GAMs was fitted to investigate the link between temporal changes in macroinvertebrate assemblages and river type in greater detail – whether trends differed between river types or – equivalently – how the effect of an environmental variable such as

catchment land cover might change through time. These models were similar to those used for temporal trends, but differed in two ways: i) a nonlinear interaction term was fitted between sampling year and the focal environmental variable, using a full tensor product smooth of cubic regression splines (Wood 2011), with the degrees of freedom of the two constituent splines fixed at nine (Fewster *et al.* 2000), and ii) no post-stratification weighting was used because the focus was not on estimating unbiased national averages. Models were fitted for five environmental variables: urban, arable and improved grassland catchment land cover, altitude and catchment area. The relationships were visualised using perspective plots and predictions of temporal trends at 'low' and 'high' values of the environmental variable (1<sup>st</sup> and 99<sup>th</sup> percentiles; Harrell 2001).

# 3.4 Results

# 3.4.1 Trends in invertebrate assemblages

Macroinvertebrate richness increased by 9 % over the period 1992-2018, equivalent to an additional 1.5 taxa on average (17.3 to 18.8 taxa; Figure 3.2a). The greatest increases occurred in the years 1991-1995 and 2015-2019, with little change in between. In 1995 there was a significant inflection in the trend, marking the end of the initial period of increase, although the post-2015 inflection was not significant.

The first axis from the correspondence analysis (CA1<sub>PA</sub>) represented the major gradient in macroinvertebrate communities, from those containing greater proportions of taxa associated with poorer water quality, slower flows and siltier substrata, such as Oligochaeta and Mollusca (negative CA1<sub>PA</sub> values; Appendix B Figure B.2), to communities characterised by taxa typical of better water quality, faster flows and greater oxygenation, such as families from the Ephemeroptera, Plecoptera and Trichoptera (positive scores). CA1<sub>PA</sub> increased across the study period (Figure 3.2b), indicating greater prevalence of taxa associated with better water quality. There were three relatively clear peaks and troughs around the overall trend before c. 2014, each associated with significant turning points. Re-running the analysis using macroinvertebrate abundance data (CA1<sub>Ab</sub>; Appendix B Figure B.2 and B.3) resulted in a similar overall increase and change in composition, although the timing of the peaks and troughs differed slightly from CA1<sub>PA</sub>. Compared to 'naïve' estimates that ignored potential biases arising from the non-random selection of sampling sites, the post-stratified trends

indicated smaller gains in both richness (1.5 taxa cf. 1.8 un-adjusted, 1992–2018) and  $CA1_{PA}$  (0.14 units cf. 0.18 un-adjusted, 1992–2018; Appendix B Figure B.4).

Functional richness declined by around 20 % over the first 20 years, reaching its lowest point around 2010 (Figure 3.2c). Similar to taxonomic richness, functional richness then increased towards the end of the time series to recover the earlier losses (Figure 3.2a and 3.2c), although there were no significant change points in the trend line.



Figure 3.2 Invertebrate trends for England and Wales in the years 1991-2019, represented by (a) invertebrate family richness, (b) CA1 scores from presence-absence data (CA1<sub>PA</sub>) and (c) functional richness. Smoothed post-stratified estimate displayed as solid black line, with dotted lines and shaded area indicating 95% confidence limits, and black points denote annual (unsmoothed) point estimates. Coloured dots along the smoothed line represent statistically significant inflections in the gradient of the curve: red indicating reduced rate of increase or greater rate of decline, and blue indicting greater rate of increase or reduced rate of decrease.

# 3.4.2 Beta-diversity

Across England and Wales as a whole, macroinvertebrate communities became more homogenous through time, although the effect was small. The main change occurred in the first part of the time series, with mean Jaccard dissimilarity declining from 0.505 in 1991-2000, to 0.480 and 0.478 in 2001-2010 and 2011-2019 respectively (Figure 3.3a).



Figure 3.3 a) Community similarity of invertebrate families in samples collected in the years 1991-2000, 2001-2010 and 2011-2019 measured as mean Jaccard dissimilarity. b) Mean invertebrate family turnover in the years 1991-2019. Total family turnover (blue line) is made up of family appearances (green) and disappearances (amber). Relationship between mean invertebrate family turnover and year (1991 -2019) based on linear regression (dashed line).

On average, 35–40 % of invertebrate families differed between samples in consecutive years at individual sampling sites (Figure 3.3b). Gains and losses occurred at similar rates, consistent with much of this turnover being a sampling effect generated by the low capture probabilities for rarer taxa in individual samples. More importantly, the rate of turnover declined at around 1 % per decade (Figure 3.3b;  $r^2 = 0.456$ , p = <0.001), indicating a gradual increase in persistence (as understood by Pimm 1984) – a facet of ecological stability. Interannual losses showed no overall trend ( $r^2 = 0.006$ , p > 0.69), whereas gains declined through the time period ( $r^2 = 0.38$ , p = <0.001), explaining the higher turnover in the 1990s.

# 3.4.3 Community change in response to catchment characteristics

Temporal changes in invertebrate communities differed between rural and urban catchments (Figure 5; Appendix B Figure B.5). More heavily urbanised catchments had lower CA1<sub>PA</sub> scores and taxonomic richness throughout, but the gap to rural rivers narrowed through time as the most heavily urbanised catchments gained ~6 families (cf. ~2 in rural catchments; Figure 3.4a) and showed slightly larger increases in CA1 (Figure 3.4b; Appendix B Figure B.5). Increases in both richness and composition continued until 2019 in urban catchments, whereas rural rivers showed little change after 2010.

Agricultural catchments dominated by arable or improved grassland land cover had a lower prevalence of pollution-sensitive taxa than catchments made up of less agricultural land cover, although richness was similar to less agriculturally managed catchments (Appendix B Figure B.5). Richness and CA1<sub>PA</sub> increased at both low and high values of arable and improved grassland landcover, although changes appeared more variable through time for richness (Appendix B Figure B.5). Richness gains were greater in less agricultural catchments, while CA1<sub>PA</sub> increased slightly more within the least agricultural catchments. Richness and CA1<sub>PA</sub> varied more through time at higher than lower altitude locations, but there was little difference in net change between 1991–2019 (Appendix B Figure B.5). Increases in CA1 were greater in larger rivers, with big gains pre-2000, whilst smaller catchments gained taxa steadily across the study period (Appendix B Figure B.5).



Figure 3.4 Smoothed temporal trends in (a) invertebrate taxonomic richness, and (b) CA1<sub>PA</sub> in the years 1991 – 2019, across a gradient of catchment urbanisation. Catchment urbanisation is calculated from the 2007 land cover map, and represented as a%. Yellow indicates higher richness and CA1 score, blue indicates lower richness and CA1 score.

b.

a.

# 3.5 Discussion

National assessments of long-term macroinvertebrate trends are only possible by extensive riverine monitoring over decadal timescales. While studies within individual river systems are valuable, analysing the status of rivers nationally allows conclusions to be drawn at policy-relevant scales, although may be characterised by distinct challenges. In the case of England and Wales, data collected in the 1990s and early 2000s often have limited taxonomic resolution (i.e. family-level) and sampling has traditionally been biased towards particular river types, for example under-representing headwaters (Appendix B Figure B.1). With careful site selection and statistical methods to minimise bias, however, it should be possible to provide more accurate assessments of current status and temporal trends, as done for England and Wales. This, in turn, adds important evidence to the ongoing debates around the status of freshwaters and macroinvertebrates in the UK, across Europe and globally (Baranov *et al.* 2020; van Klink *et al.* 2020; Outhwaite *et al.* 2020; Stepanian *et al.* 2020).

This study revealed the dynamic nature of English and Welsh rivers over three decades, most notably a 9 % increase in macroinvertebrate richness (1992-2018) and a large shift towards families characteristic of better water quality. Contrary to Prediction 1, gains of pollutionsensitive taxa continued across the full time period, whereas gains in richness stalled around 1995-2015, consistent with the prediction, before showing some evidence of an increase post-2015. This increase was not evident in an earlier analysis of English rivers (Pharaoh et al. 2021), perhaps indicating that improvements within Welsh rivers may lag behind those in England. Despite the ongoing changes, the turnover rate declined steadily – community composition became more consistent through time. Increases in richness and CA1 continued up to 2019 in heavily urbanised catchments, fitting with Prediction 2. Moreover, consistent with the predictions, the macroinvertebrate communities in different rivers became more homogenous over time. Increasing homogeneity, trends in richness and CA1 scores, alongside the greatest changes in the most urbanised catchments are consistent with water quality improvements in the most polluted locations narrowing the gap in quality to more rural rivers. The impact of urbanisation on rivers is well studied, and urban rivers have long been characterised by macroinvertebrate communities made up of species adapted to less favourable conditions (Roy et al. 2003; Walsh et al. 2005; Chadwick et al. 2006; Urban et al.

2006). Long-term improvement in these rivers indicate diminishing pressures through time, driven by legislation targeted at urban areas (e.g. the Urban Wastewater Treatment Directive) which have led to substantial reductions in organic pollution (Council of European Communities 1991; Bowes *et al.* 2005; Whelan *et al.* 2022). Despite this, less urban catchments continue to support higher richness and a greater proportion of pollution sensitive taxa than their urban counterparts.

Our results for taxonomic richness and composition are consistent with an earlier analysis of national monitoring data covering 1991-2008 (Vaughan and Ormerod, 2012). Although the estimated increase in richness in the current study is smaller than that estimated in the 2012 study (9 % v. 20 %), direct comparison of these values is not appropriate due to methodological differences. This study excludes the first and last years value from calculated percentage change, as GAMs provide less reliable estimates at the ends of the time series (Hewson and Noble 2009). This produces a more conservative figure, whereas if these years were included a 15 % increase would be reported. Despite a shared geographical coverage and similar post-stratification procedures, differences in methodology and site selection further complicate these comparisons. Due to the inclusion of more sample sites in this analysis and post-stratification using the second RHS baseline, which better represented smaller headwater streams, this new analysis should be more representative of English and Welsh rivers as a whole (Seager et al. 2012). The changes in richness are also consistent with an analysis of freshwater invertebrate data from UK recording schemes and societies covering 1970–2015 (Outhwaite et al. 2020). This found a rapid increase in the prevalence of many freshwater taxa in the early 1990s, which appeared to plateau around 2005. Notably, Outhwaite et al. (2020) revealed a large-scale decline prior to the 1990s, placing the more recent changes in a wider context and emphasising the importance of the choice of baseline year when interpreting long-term trends (Baranov et al. 2020). Macroinvertebrates respond rapidly to changing environmental conditions (Vaughan and Gotelli 2019), suggesting that these historical changes were responses to contemporaneous changes in the river environment (e.g. water quality and land use).

The contrasting trends between richness and composition (CA1<sub>PA</sub>) re-emphasise the importance of using multiple measures to assess biodiversity trends, as single metrics such as taxon richness may overlook more subtle changes (Larsen *et al.* 2018). Despite little change in overall richness through the mid-portion of the time series, there was a large change in composition, reflecting a mixture of families increasing and decreasing. Composition may also be more sensitive to climatic variation: the fluctuations observed in CA1 (cf. richness) around the overall trends are likely attributable to climatic variability, such as that captured by the North Atlantic Oscillation index (Bradley and Ormerod 2001; Vaughan and Ormerod 2014), and further highlight the dynamism of macroinvertebrate communities.

Functional and family-level richness showed some similar changes through time, but functional trends did not show a net increase between 1991 and 2019. Instead, functional richness declined through the mid-portion of the time series (c. 1995–2010), during the same period when taxonomic richness was largely unchanged. This suggests that while family richness was maintained, the turnover of families present led to a reduction in niche breadth, as species with distinctive functional traits were lost. This may be an example where functional diversity measures are more sensitive than taxonomic ones to human activities (Mouillot et al. 2013) or it may result from lower functional diversity amongst pollution sensitive taxa, such that in the absence of gains in taxonomic richness, functional richness declined. Functional richness is often considered to reflect the ways through which changes in species may alter ecosystem functioning. In the case of macroinvertebrates, this includes freshwater macroinvertebrates providing functions such as detritus processing and energy transfer to consumers (Wallace and Webster 1996; Abonyi et al. 2018). This functional approach to assessing changes in river communities also allows a perspective on how changing conditions could affect river ecosystem services (Macadam and Stockan 2015).

Invertebrate communities in predominantly agricultural catchments, both arable and improved grassland, were typical of poorer water quality, similar to heavily urbanised catchments. Temporal changes in invertebrate communities differed across the agricultural land use gradients, suggesting that agricultural impacts did not remain consistent through time, akin to urbanisation. Agriculture is cited as one of the most common reasons

preventing rivers from achieving good status in the UK (Environmental Agency 2021; House of Commons Environmental Audit Committee 2022), but its impacts will vary with land management practices, stocking type/density, agrochemical use and other factors that warrant further research (Hooda *et al.* 2000; Wijesiri *et al.* 2018). Quantifying agricultural intensity is a challenge and needs to move beyond the simple measures of catchment land cover used here. Specifically, some agricultural practices, such as intensive livestock units which have become increasingly controversial (Caffyn 2021), would not be reflected by assessments involving catchment land cover.

Although evidence gathered in this study can help to appraise actions aimed at improving the status of rivers, to identify the drivers in detail and better understand the interactions among multiple stressors, further work is required. The major role of catchment land use, and how its importance may be changing through time, has been highlighted here, but understanding the proximate mechanisms by which it impacts organisms is needed for developing management prescriptions. Other potential drivers need also to be considered, including invasive non-native species such as the signal crayfish (*Pacifastacus leniusculus*) which is prolific across Great Britain and has impacts on both physical habitat and direct biotic interactions with other macroinvertebrates (Holdich et al. 2014; Mathers et al. 2016a). Furthermore, national-scale increases in richness and gains in pollution-sensitive taxa may disguise localised declines or those associated with particular river types, such as some upland rivers or chalk streams (Vaughan and Ormerod, 2012; O'Neil and Hughes, 2014) Research into what is driving trends across these areas will be essential for identifying locations where a more targeted approach to achieve biological recovery could be utilised. This will be of particular use in an era of changing environmental policy and legislation (Burns et al. 2019; Klaar et al. 2020).

# 3.6 Conclusion

Results are consistent with biological recovery of English and Welsh rivers continuing over 30 years. Recovery was greatest within urban rivers, where improvements in water quality and biota have been well documented, but improvements were also observed in rural catchments. This highlights the need for a better understanding of the drivers of change in rivers to identify potential management interventions to improve ecological quality further. At the same time, previous work has demonstrated regional differences in macroinvertebrate trends beyond simple land cover relationships (Vaughan and Ormerod, 2012), and it is likely that the national trends presented here disguise localised declines. Together, these represent two research priorities to improve our understanding of national scale changes in river quality.

# Chapter 4: Spatial patterns of changes in river invertebrate communities and their potential drivers

# 4.1 Summary

River invertebrate communities have shown recovery from organic pollution across England and Wales over recent decades. Analyses of complex systems such as these can, however, result in oversimplification and bias when multiple temporal and spatial factors are not considered simultaneously. Despite evidence of recovery, there remains a key need to assess how trends vary across landscapes, and to identify the factors responsible for changes in order to support policy and management decisions. Attributing changes in invertebrate communities to specific environmental conditions at large scales is challenging, since relationships vary across space, and the individual effects of variables are difficult to disentangle from one another. The aims of this chapter are to: i) map changes in river invertebrate richness and community structure across England and Wales using data between 1991 and 2019, and ii) attribute changes to environmental variables (water quality, water temperature and catchment land cover). This analysis utilises structural equation models (SEM) to capture potentially complex relationships between environmental variables, and geographically weighted regression models (GWR), which relax the assumption that relationships are constant across space. Results reveal that invertebrate richness and the prevalence of pollution-sensitive taxa (signified by increases in CA1 score) increased across much of England and Wales, but declines occurred in some locations. Notably there were declines in the prevalence of pollution sensitive taxa (declines in CA1 score) in some upland locations. Trends were not spatially consistent between invertebrate measures (e.g. richness declined in south-west Wales and to the north-west of London, while pollution-sensitive taxa increased within these areas). Geographically weighted regression models showed that the effects of water quality variables and land use varied across space and time. For example, nitrate and family richness were negatively related at the start of the time series in the south of Wales, but positively related at the end of the time series. Whilst nitrate's relationship with CA1 score was negative at both the start and the end of the series in the same area. Arable land had the most varied coefficients across space for CA1 score and richness, reflecting both strong negative and positive relationships.

SEMs identified relationships between water quality variables and invertebrate measures, with the greatest negative relationship between phosphate and CA1 score. Land use had direct and indirect effects, with the greatest direct relationship being a negative relationship between urbanisation and richness. Water quality measures were also interrelated, for example, water temperature had a negative relationship with biochemical oxygen demand.

Overall, the results highlight the importance of spatially explicit analyses, revealing diverse changes that simple national trends (Chapter 3) can overlook. This investigation complements analysis of trends across land use, as it reveals that even relationships between invertebrate measures and land use vary across space and time. Using GWR and SEM together highlights how investigations of trends across large areas through time without consideration of spatial effects can overlook important details.

# 4.2 Introduction

Large-scale assessments are vital in determining status and trends in biodiversity. In particular, biodiversity trends estimated at national or international scales can be powerful tools to catalyse conservation actions and inform policy. Examples include the Living Planet Index, which appraised trends in global biodiversity based on vertebrate populations. This was subsequently adopted by the Convention of Biological Diversity (CBD) as an indicator of progress towards its targets, including the effects of the post-2020 Global Biodiversity Framework to 2030 (Secretariat of the CBD, 2020). At the continental scale, North American bird population trajectories have been used to investigate trends over 48 years from the 1970s (Rosenberg *et al.* 2019). The UK uses a set of indicators to report on its progress towards meeting targets and goals set out in the Strategic Plan for Biodiversity as part of its reporting requirements as a signatory to the CBD (JNCC 2019; DEFRA 2021). This includes the monitoring and assessment of trends in bats, birds, and butterflies (Fox *et al.* 2015; Bat Conservation Trust 2021; DEFRA 2021; Harris *et al.* 2022).

While these global or national trends are of major importance, there is a risk that such coarse-scale assessments might mask or hide finer-scale trends that could have implications on local biodiversity or require specific stakeholder actions (Levin 1992; McGill *et al.* 2015). In the same way, national-scale analyses of potential drivers of change may overlook more

localised relationships between species and environmental variables that are a major source of ecological complexity and an opportunity for management intervention (Lawton 1999).

UK freshwaters have been some of the most intensely monitored ecosystems over recent decades, through a combination of statutory river monitoring (e.g. Water Framework Directive (WFD)) and voluntary recording schemes (e.g. Riverfly Recording Schemes). This has allowed powerful analyses of national trends and has revealed a largely positive picture of biological recovery from poor water quality across England and Wales over the last 30 years, despite nuanced trends in water quality (Vaughan and Ormerod, 2012; Pharaoh, Ormerod and Vaughan, 2021, 2021; Whelan et al. 2022). There has, however, been little attempt to disaggregate national trends (Qu et al. 2023), leading to a disconnect between national trends and more detailed, local-scale studies and WFD assessments (Mawle 2021; Environment Agency 2022a). For example, an initial analysis by Vaughan and Ormerod (2012) highlighted some areas in England and Wales where changes between 1991 and 2008 did not match the national story of recovery from historical water quality problems. In this work, taxon richness was observed to be declining in parts of Wales and southern England, whilst a shift towards taxa characteristic of poorer water quality was also observed in upland Wales and northern England. This initial assessment has since remained largely unexplored.

Land use, land management type and other catchment characteristics (e.g. altitude, geology) can all alter water quality (Allan 2004; Jarvie *et al.* 2008; Rothwell *et al.* 2010b; Rothwell *et al.* 2010a), in turn affecting river invertebrates through their varying tolerances to environmental conditions. Attributing changing invertebrate communities to specific environmental conditions at a national scale brings challenges, particularly when working with spatial and temporal variation in environmental variables, invertebrate communities, and their interconnections. While some variables are static through time but fluctuate across space (e.g. altitude, geology), other variables fluctuate through time and space (e.g. temperature, water quality, hydrology, land use). These variables are often altered by anthropogenic activities and are known to drive changes in invertebrate communities through time, making invertebrates a well-established tool for assessing environmental conditions (Cairns and Pratt 1993). In addition, rivers are simultaneously affected by multiple stressors, which may interact in complex ways. In some instances this can lead to

combined effects that are greater than the sum of the individual stressors (synergistic effects), while the opposite is true of antagonistic interactions (Folt *et al.* 1999; Birk *et al.* 2020). Some studies, for example, have found nutrient enrichment to worsen negative effects of fine sediment pollution on invertebrates (Townsend *et al.* 2008; Wagenhoff *et al.* 2012). The mix of stressors will vary across the UK, reflecting large-scale changes in factors such as climatic conditions and catchment land cover. Impacts of stressors on rivers can also be mitigated or exacerbated by catchment and river characteristics, and land management practices. For example, soil texture and slope modulate the risk of soil erosion from agricultural land, altering the rate of fine sediment addition to rivers (Boardman 2015; Wilkes *et al.* 2019; Boardman *et al.* 2020). Similarly, factors such as bedrock geology (e.g. limestone) can mitigate river acidification by acting in a buffering capacity (Sullivan *et al.* 2007; Harpold *et al.* 2010). Therefore, invertebrate communities can be influenced by catchment activities, the effects of which can vary across the geographical extent of the UK.

Work on rivers at the national scale in England and Wales (1991 – 2019) has linked increases in richness and shifts to taxa characteristic of better water quality to land use, with the greatest improvements occurring in the most urban areas (Environment Agency, 2021; Chapter 3). Differences among rivers have been attributed to a combination of natural gradients and anthropogenic pressures, while temporal changes were correlated with improving water quality and discharge. Other work by Vaughan and Ormerod (2014) identified a correlation between overall changes in invertebrate taxon prevalence (1991 – 2011) with pollution sensitivity and discharge. Short-term variations in prevalence correlated with temperature and nutrient concentration, while in the longer-term this was only correlated with water quality. At the same scale over 1990 – 2000, Monk *et al.* (2008) found that river flow variability influenced macroinvertebrate community response (LIFE score).

Within England in the years 2002 – 2019, research by Powell *et al.* (2023) found that trends in river invertebrate abundance across different typologies (based on dominant geology and catchment altitude) were heterogeneous. Stronger positive trends in abundance were found in organic lowland rivers across taxa and trophic groups. In contrast, the greatest declines were found in siliceous lowland rivers. Within England, analysis across an extended period identified strong relationships between the environment and community composition

(Pharaoh *et al.* 2021). The degree to which changes though time and space could be attested to changing water quality, temperature and discharge was, however, uncertain. The relationships between invertebrate communities and environmental predictors across large spatial extents are likely to be non-stationary, differing in strength or direction across regions (da Silva Cassemiro *et al.* 2007). Prior work investigating spatiotemporal drivers of UK river invertebrate communities has identified that variation among locations is greater than variation through time (Vaughan and Ormerod, 2012; Pharaoh, Ormerod and Vaughan, 2021), indicating a need to establish exactly how they vary across space

Previous work linking riverine communities to environmental variables at large scales has tended to use regression or closely related approaches (Clarke *et al.* 2003; Monk *et al.* 2008; Birk *et al.* 2020; Dobson *et al.* 2022). These have revealed connections between land use, water quality and community structure. Community responses to flow regime dynamics and responses of freshwater biota to a range of stressors have also been revealed by these methodologies, which underpin standard biomonitoring tools. Traditional regression approaches do, however, suffer from two limitations: i) a limited ability to handle complex interactions between multiple variables, and ii) the difficulty of analysing local or regional variations in the relationships between communities and their environment. This may disguise or confound important relationships and make it harder to identify the underlying causes of biodiversity change.

Two methods which have the potential to overcome this are structural equation modelling (SEM) and geographically weighted regression (GWR). Structural equation modelling has a greater ability to handle collinearity and reveal causal relationships compared to conventional regression methods (Shipley 2016). Recent examples of its use in ecology include work revealing how species interactions and habitat attributes could be misconstrued as direct consequences of a foundation species, like giant kelp in rocky reef communities (Miller *et al.* 2018). They have also been used to distinguish direct and indirect abiotic influences on algae density in shallow eutrophic lakes (Chao *et al.* 2022) and grassland plant and invertebrate species richness in a lowland agricultural field trial site (Gillespie *et al.* 2022). Geographically weighted regression modelling allows regression coefficients to vary geographically, enabling analyses that explicitly tackle the issue of local variation in relationships between environmental variables and ecological responses

(Osborne *et al.* 2007). This has potential to be a powerful exploratory tool for dealing with complex relationships at large scales. Examples of its use include investigation of spatial relationships between freshwater acidification critical load and catchment data across Great Britain (Harris *et al.* 2010) and identifying geographic variation in the relationship between large scale environmental variables and global bat species richness (Alves *et al.* 2018). By combining these two approaches, new insights could be given into what is driving changes in river communities and identifying how this may vary across space.

Using data from the years 1991 – 2019 inclusive and a core set of 3982 locations from across England and Wales (which are subset appropriately to conduct each analysis within this Chapter – see Methods), this study aims to identify how invertebrate trends as well as their relationships with land use and water quality vary across England and Wales. It also seeks to identify direct and indirect relationships between these variables and invertebrate communities. Specific aims for this study are threefold: i) to disaggregate changes in river invertebrate communities over 29 years (1991-2019; Chapter 3) across England and Wales, to identify areas that have not followed the overall trend of improvement; ii) to use GWR to investigate how relationships between water quality, land use and invertebrate communities varied across England and Wales, and if this varied through time (1991 – 2004 v. 2005 – 2019); and finally, iii) to use SEM to link landscape and water quality variables to invertebrate communities by identifying direct and indirect mechanistic relationships between these variables and invertebrate communities. It was predicted that:

- Hypothesis 1: Larger increases in taxonomic richness and the prevalence of pollutionsensitive taxa will occur in urban areas, whilst declines will occur in the uplands, as informed by previous research (Vaughan and Ormerod, 2012). Increases will be more widespread than declines.
- Hypothesis 2: Relationships between invertebrate communities with water quality and land use will vary across England and Wales, reflecting idiosyncrasies of different catchments and river types. These spatial differences will remain constant between the two time periods.
- Hypothesis 3: Water quality variables (nitrate, phosphate, biochemical oxygen demand (BOD), temperature and pH) and discharge will have larger measured impacts on

invertebrate communities than land use. Catchment urbanisation will have both direct and indirect effects on invertebrate communities.

#### 4.3 Methods

#### 4.3.1 Data preparation

#### 4.3.1.1 Macroinvertebrate data

Environment Agency and Natural Resources Wales macroinvertebrate data covering 29 years were used (1991 – 2019 inclusive), representing 47009 samples from 3982 sites (see Chapter 3 for details of data selection and processing). Invertebrate family richness and the scores from the first axis of a correspondence analysis (CA1 score) calculated from macroinvertebrate presence-absence data were obtained for each sample. Negative CA1 scores represented communities with greater proportions of taxa associated with poorer water quality, slower flow, and siltier substrata (e.g. Oligochaeta and Mollusca) while positive scores were indicative of taxa typical of better water quality, faster flows, and greater oxygenation (e.g. Ephemeroptera, Plecoptera and Trichoptera; Chapter 3; Appendix B Figure B.2). Correspondence analysis loadings for the 78 composite and family groups (Appendix B Figure B.2) were positively correlated with Biological Monitoring Working Party (BMWP) taxon scores (Hawkes 1998), confirming that increasing CA1 scores reflected the increasing prevalence of pollution sensitive taxa (Spearman rho = 0.48, p = <0.001).

The catchment was delineated for each macroinvertebrate site (see Chapter 3), and the percentage of urban or suburban land cover, improved grassland or arable agriculture were calculated from a 1 km resolution UK Land Cover Map 2007 (Morton *et al.* 2014). Land cover has changed across much of the UK over this period, with the exception of higher altitude areas which have remained relatively constant in their land use (Land Cover Change dataset Great Britain, 1990-2015). Land cover maps are, however, not suitable to measure changes through time due to differences in methodology. Whilst available land cover change maps (1990 – 2015) are suitable for comparisons through time, they rely on simplified classes. For example, instead of improved grassland, rough grassland and calcareous grassland in individual years, the land cover change map combines all grassland together. This prevents direct comparisons with Chapter 3 (and other related analysis) which focusses on improved grassland and would divert attention away from more intensive land use types which are

deemed higher risk for nutrient losses. Therefore, a single value from the land cover map closest to the middle of the time series (2007) was used.

#### 4.3.1.2 Environmental data

For GWR and SEM, a subset of the full 3982 locations was selected where macroinvertebrate sampling locations could be matched to nearby water quality sampling locations and gauging stations. Water quality data covering 1990 – 2019 were sourced from routine monitoring data from the Environment Agency and Natural Resources Wales. Annual medians for pH, temperature, BOD, orthophosphate and either total organic nitrogen (TON) or nitrate were calculated for the 12 months prior to the spring macroinvertebrate sampling period (March - May). Medians were calculated where at least nine out of the 12 calendar months were sampled with all determinands present. Where sampling occurred more frequently than once a month, a maximum of two randomly selected samples per calendar month were retained to minimise bias towards seasons more frequently sampled. Where ≥50 % of values were below detection limits, regression-onorder-statistics was used to estimate medians using the NADA package (Lee and Helsel, 2005): this, and all subsequent statistical analyses, were carried out using R version 4 (R Core Team, 2021; R Studio Team, 2021). Missing nitrate values were predicted from TON by fitting a linear regression to predict nitrate from TON at locations where both determinands were recorded. Predictions for nitrate were then generated where only TON was recorded (Vaughan and Ormerod, 2012). Annual medians are widely used when studying long term trends in invertebrate communities and water quality variables. (Vaughan and Ormerod 2012; Peters et al. 2014). Annual medians are skewed less by extreme values, which are expected when working with data of this nature, leading to a more robust depiction of the central tendency of the data. Despite the compromise of detail using this methodology, such as including years where each month is not sampled, the number of sites which are retained is maximised. This is advantageous when working with coarser national monitoring data, where data collection has not been designed specifically for its use in research studies.

Discharge data for 1990 – 2019 were accessed from the UK National River Flow Archive (nrfa.ceh.ac.uk). The median daily discharge for the 12 months prior to the spring sampling period was calculated. Discharge was divided by gauging station catchment area to provide a measure of discharge that is independent of catchment area (units =  $m^3 s^{-1} km^{-2}$ ), a

measure which is used across the hydrological and ecological literature (Schmocker-Fackel *et al.* 2007; Coleman *et al.* 2011). Reporting discharge in this manner ensures that the discharge is not just a reflection of the catchment size, which would be heavily influenced in this dataset where sites range from upland to lowlands.

The 3982 macroinvertebrate sites were paired to the nearest water quality sites and gauging stations. Macroinvertebrate sites were removed where no match was found within 1 km for water chemistry locations and 5 km to a gauging station (Vaughan and Ormerod, 2012). These different criteria reflected the greater density of chemical sampling points compared to gauging stations and provided a good compromise between close spatial correspondence between different data sets and maximising the resulting sample size. Data were filtered so that only years which contained macroinvertebrate, chemistry and discharge data were retained. This resulted in a final sample size of 672 macroinvertebrate locations, with a mean of 5.4 years samples per site (Figure 4.1).



Figure 4.1 Subset of invertebrate biology sites (n=672) paired with water quality sites and gauging stations.

#### 4.3.2 Spatial patterns of invertebrate communities

Geostatistical interpolation was used to estimate family richness and CA1 score across England and Wales at three time points to calculate how invertebrate communities changed spatially through time. To maximise spatial coverage of sites for interpolation, invertebrate data were pooled in 1991 – 1993, 2004 – 2006 and 2017 – 2019. Mean values were calculated for any sites sampled more than one year in each window, resulting in sample sizes of 2639, 3819 and 1664 for the three time windows, respectively. Ordinary kriging using the gstat package in R was used to estimate richness for a 10 km grid of England and Wales, which reduced processing time while estimating values at a spatially relevant scale (Pebesma 2004). Despite pooled years, coverage of some areas in the north-west and southwest England in the years 1991 – 1993 was poor. Consequently, maps were clipped to exclude areas which fell more than 20 km from the nearest invertebrate site for the 1991 – 1993 time period.

To test Hypothesis 1, richness and CA1 score changes between 1991 – 93 and 2017 – 19 were calculated from interpolated maps using the raster calculator in QGIS, by subtracting the later interpolated maps from earlier years (regions not included in 1991 – 93 maps were also not included in change plots).

#### 4.3.3 Geographically weighted regression

Geographically weighted regression modelling was used to investigate how relationships between water quality and land use with invertebrate communities varied across England and Wales, to test Hypothesis 2. Models were fitted for two time periods, 1991 – 2004 and 2005 – 2019, to assess whether these relationships varied across time. Geographically weighted regression modelling is a non-parametric multivariate modelling technique which can be used to identify how locally weighted regression coefficients vary across a study area (Fotheringham *et al.* 2003). Coefficients in the model are specific to each location and are based on observations taken at sample points near to that location which are geographically weighted using a distance function up to a given bandwidth (analysis extent). The resulting map of estimated coefficients revealed how relationships between environmental variables and invertebrate communities varied across the country.

To ensure consistency in spatial coverage between the two time periods, only sites which were present in both halves of the time series were retained from the paired water quality and invertebrate biology data, leaving a total of 465 locations. For variables which were not constant through time (pH, temperature, BOD, nitrate, phosphate, richness and CA1 score), mean values for each site within both periods were calculated. Water quality and land use data were transformed using the bestNormalize package in R (Peterson 2021). A correlation matrix was produced using the R package corrplot to visualise correlations between independent variables (Wei *et al.* 2017; Appendix C Figure C.1). As no correlation coefficients were > 0.7, it was determined that multicollinearity was sufficiently low to retain all variables (Dormann *et al.* 2013).

Four GWRs were fitted using the spgwr package (Bivand *et al.* 2022): separate models for the first (1991 – 2004) and second halves of the time series (2005 – 2019) for both invertebrate richness and CA1 score. Explanatory variables were water pH, temperature, BOD, nitrate, phosphate, and catchment improved grassland, arable and urban land cover percentage. Prior to fitting the GWRs, conventional linear regressions were fitted and the residuals assessed to check that a Gaussian error distribution was appropriate. The optimal bandwidth for GWR was determined using an adaptive kernel function with cross validation, using the gwr.sel function (Bivand *et al.* 2022). Using an adaptive bandwidth, kernels vary in size across the study area, dependent on the local data density: where data are sparse kernels have larger bandwidths, and have smaller bandwidth where sample site density is higher. Geographically weighted regressions were then fitted using the selected bandwidths. Quasi-global  $r^2$  was taken from each model summary, which describes the percentage of invertebrate variation explained by environmental variables. Local  $r^2$  for sites within each model was also reported.

Mean coefficients in the two time periods for each environmental variable were calculated. Violin plots were produced in ggplot2 to display the density of the data at the range of coefficients, and to display how the distribution of coefficients varied between time periods.

# 4.3.4 Water quality change through time

To provide context for the GWR outputs, maps of the five water chemistry determinands were generated following the same basic approach as maps of richness and CA1 above.

Water quality data were pooled within 1991 – 2004 and 2005 – 2019 to match the GWR timeframes, and mean values were calculated from the annual averages at each site. Ordinary kriging was then used to estimate water chemistry across the 10 km grid of England and Wales, and changes through time calculated by subtracting the earlier interpolated maps from later years.

## 4.3.5 Structural equation modelling

Structural equation models were constructed to identify direct and indirect mechanistic relationships between landscape and water quality variables, and invertebrate communities, to test Hypothesis 3. Indirect relationships are those where one variable may influence another thorough an effect mediated by another variable. For example, rainfall may influence invertebrate communities through its effect diluting pollutants within a waterbody. Structural equation models allow variables to be used as both predictors and response variables within the same model (Kline 2016). This has been credited as a valuable tool particularly for understanding cascading effects, such as those from land use to water. Examples of their use include investigating relationships between land use characteristics, fertiliser and manure application within a catchment, total nitrate concentration and submersed aquatic vegetation in Chesapeake Bay (Lefcheck *et al.* 2018). SEMs were fitted to the 672 invertebrate sites with invertebrate and environmental data remaining at annual resolution, rather than pooling within time windows.

Structural equation model analysis can be compromised if causal variables are too highly correlated, therefore a correlation matrix was produced using the R package correlation visualise correlations between independent variables (Appendix C Figure C.2; Wei *et al.* 2017). As no correlation coefficients were > 0.7, it was determined that this was sufficiently low that multicollinearity between variables would not be an issue in the analysis (Dormann *et al.* 2013). To investigate the influences of water quality, river discharge and land use on invertebrate communities, the model included: pH, temperature, BOD, nitrate, phosphate, median discharge, urbanisation and improved grassland and arable land. To meet the normality assumption of SEM, data were transformed (specific transformations for each variable are presented in Appendix C Table C.2)

A conceptual model was constructed that linked water quality, land cover and climate variables to family richness and CA1 score (Figure 4.2). This represented hypothesised direct links to the response variable (e.g. BOD and phosphorous linked to CA1 score) and indirect links mediated via other environmental variables (e.g. urbanisation linked to BOD, in turn linked to CA1 score).

The initial model was based on the following equations, broken down from the conceptual model (Figure 4.2). Each of these models displays the response variable and the other variables it was directly hypothesised to be linked to.

- Nitrate <- Urbanisation % + Improved grassland % + Arable % + Scaled median discharge
- Phosphate <- Urbanisation % + Improved grassland % + Arable % + Scaled median discharge</li>
- iii. pH <- Urbanisation % + Improved grassland % + Arable % + Scaled median discharge
- Temperature <- Urbanisation % + Improved grassland % + Arable % + Scaled median discharge</li>
- v. BOD <- Temperature + Phosphate + Urbanisation % + Improved grassland % + Arable</li>
  % + Scaled median discharge
- vi. CA1 score or family richness <- Nitrate + Phosphate + pH + Temperature + BOD +</li>
  Scaled median discharge + Urbanisation % + Improved grassland % + Arable %

The first four equations (i. – iv.) describe the hypothesised effects of catchment land cover (urban, arable and improved grassland) as well as the effects of discharge on the concentration of nitrate, phosphate, pH and temperature of water. This was intended to capture the known impacts of land cover on water quality, in addition to the potential dilution effects of discharge. Equation v. included these same predictors, alongside temperature and phosphate which were expected to influence BOD (Mallin and Cahoon 2020; Chapra *et al.* 2021). Equation vi. encapsulated all the hypothesised direct drivers of CA1 score and richness, with each of the water quality measures, land use and discharge.



Figure 4.2 Conceptual model for direct and indirect factors influencing invertebrate richness and CA1 score. Colours of arrows distinguish direct paths for each variable or pair of variables. For example, orange arrows indicate all hypothesised direct relationships with nitrate and pH, dark blue arrows indicate all hypothesised direct relationships for richness and CA1 score.

Structural equation models were constructed using the piecewiseSEM package (Lefcheck 2016) because it is compatible with repeated measures (longitudinal) data and hierarchical study designs. Within the SEM, each response variable was modelled using a linear mixed-effects model (LMM) using the nlme package (Pinheiro *et al.* 2022) to represent equations i-vi. Therefore, the model is comprised of multiple LMMs nested within the SEM. Linear mixed-effects models were fitted with biological sample site and year as random effects, to address non-independence of sites and temporal autocorrelation (Lefcheck *et al.* 2018). The need for a residual temporal autocorrelation term was checked by applying a first-order autoregressive (AR1) correlation structure (Lefcheck 2016), which is compatible with unevenly spaced observations through time (Singer and Willett 2003). This, however, resulted in higher Akaike Information Criterion (AIC) scores, so it was not included in the final models.

From the conceptual model, paths with *p*-values which were insignificant were removed from the model individually, following methodology by Lefcheck (2016). This followed a strictly confirmatory approach, beginning with the largest *p*-value until only significant paths (p < 0.05) remained in the model. The SEM produced a range of likely meaningless independence claims, where a relationship is derived between two variables in the model without clear scientific reasoning. For example, water temperature predicting phosphate concentration. These were removed from the basis set (the smallest possible set of independence claims), and instead specified as correlated errors (Lefcheck 2016). Similarly, as piecewise SEM cannot disentangle cyclic or reciprocal relationships in the same model, the literature and knowledge of natural processes were used to exclude directional paths which are unlikely. Model fit was assessed using Fisher's C and AIC.

# 4.4 Results

# 4.4.1 Spatial invertebrate changes through time

There was large geographical variation in richness and community composition across England and Wales, and also in the changes in these two metrics over the study period (Figure 4.3). Across the time period, the highest values for richness were mostly concentrated in south-west England (Figure 4.3a). At the beginning of the time series (1991 – 1993) there were large areas of lower richness which appeared to correspond to urban centres e.g. surrounding London, Birmingham, Liverpool, Manchester and more urban areas of South Wales (Figure 4.3a; see Appendix C Figure C.3b for urban land cover map). In the north-east coast of England, an area of lower richness was apparent across the timeframe (Figure 4.3a). The highest CA1 scores, indicative of communities characterised by pollutionsensitive taxa, appeared around the north of England and central Wales. This was consistent across the time series, corresponding with upland areas (Figure 4.3b; Appendix C Figure C.3b)

Change in richness from 1991 – 1993 to 2017 – 2019 ranged from losses of eight invertebrate families to increases of 14 through time (Figure 4.3a). Across much of England and Wales, richness increased from 1991 – 93 to 2017 – 19. The greatest increases were in south-east Wales, areas to the east of London, the English Midlands and parts of northern England (Figure 4.3a). The most substantial declines occurred in the south-west of Wales, the north-east and south-west of England, the northern portion of the south-east (around Oxford) and around the southern coast of the south-east (Figure 4.3a). Among regions with the greatest declines were areas which had the highest richness at the start of the time series (south-west England and Wales). At the beginning of the time series, a large area of low richness was centred around the western Midlands, which constricted by 2004 – 2006 and further by 2017 – 19 (Figure 4.3a).

The largest CA1 score increases and declines were equal in magnitude, changing by 0.6 in both directions (Figure 4.3b). Across the majority of England and Wales CA1 score increased or remained static, with the only notable reductions around mid-Wales, and areas of the north-west of England which corresponded to higher starting values of CA1 score in 1991 – 1993 (Figure 4.3b). Changes in CA1 score did not appear to correspond with richness: CA1 score largely increased through time in locations that showed the greatest declines in richness (Figure 4.3a and 4.3b).



Figure 4.3 Kriged maps of (a) richness and (b) CA1 score for 1991-1993, 2004-2006 and 2017-2019 and change from the first to last time period. 95 Blank regions in 1991-1993 and change maps reflect areas where data were sparse and it was inappropriate to calculate invertebrate measure and change through time.

# 4.4.2 Spatial variation in relationships between water quality and land use with invertebrate communities

Geographically weighted regression results showed that the correlations between environmental variables and invertebrate measures varied greatly across England and Wales, and often between richness and CA1 (Figure 4.4 – 12). Environmental variables explained up to 64 % of variation in richness (i.e., from quasi-global  $r^2$  = 0.64 for 1991 – 2004 and 0.60 for 2005 – 2019), and 84 % for CA1 (quasi-global  $r^2$  = 0.84 in 1991 – 2004 and 0.75 in 2005 – 2019). The predictability of the invertebrate community varied markedly across England and Wales, and between invertebrate measures, based on the local  $r^2$  value (Appendix C Figure C.4). CA1 score observed a strong east-west gradient, with higher predictive power in the west and lower in the east, whilst for richness,  $r^2$  was less variable and observed different spatial trends (Appendix C Figure C.4). Slope coefficients across England and Wales varied between the time periods, with changes observed in both magnitude (e.g. a weaker relationship between BOD and CA1 score in north Wales post-2005) and sign (Figures 4.4 – 12). For example, in south Wales, the relationship between nitrate and richness was negative pre-2005 and positive post-2005 (Figure 4.4c). This changing relationship did not correspond to changes in nitrate concentrations (water quality variables in each of the time periods and their observed changes through time are included in Appendix C Figure C.5), remaining in the lowest category (0.3 - 2.8 mg l<sup>-1</sup>) in both halves. This region did not correspond to any other large changes in any other environmental variables, whilst these sites were located within the most urban region of Wales (Appendix C Figure C5).

Amongst nutrients, phosphate had predominantly negative relationships with richness and CA1 score, whereas for nitrate, positive values were estimated more frequently (Figure 4.4a - d). The negative relationships with phosphate weakened northwards for CA1 and richness, and positive coefficients were estimated in a few parts of northern England (Figure 4.4a and 4.4b). The magnitude of coefficients increased through time for richness, but decreased for CA1 (Figure 4.4a and 4.4b). For nitrate, coefficients were mostly negative in 1991 – 2004 for richness, whereas by 2005 – 2019 areas in south Wales and central and southern England were positive (Figure 4.4c). This was consistent with higher richness where there was some nutrient enrichment. In the north-east of England, coefficients became more negative

(Figure 4.4d). Relationships between nitrate and CA1 score contrasted with this, with evidence that nitrate was positively associated with greater prevalence in pollution-sensitive taxa across central and south-eastern England pre-2005, but becoming negative towards the north and west, with this positive relationship disappearing after 2004 (Figure 4.4d). Despite the variability of nitrate concentrations through time varying across the spatial extent of England and Wales, these did not appear to coincide with the changing relationships between nitrate concentrations and invertebrate measures (Appendix C Figure C.5b; Figure 4.6).

Relationships between BOD and invertebrate measures were mostly negative or neutral across the UK, except for a part of south-west England (Figure 4.5a - b). Clear gradients were evident in the coefficient estimates for both richness and CA1 but mirrored one another: coefficients became increasingly negative moving east to west for richness (Figure 4.5a), and the converse for CA1 (Figure 4.5b). Biochemical oxygen demand declined or remained constant through time across most England and Wales, with the greatest declines located in central England (Appendix C Figure C.5c). In both cases, the mean coefficient estimates became less negative through time (Appendix C Table C.3 for mean coefficients).

Coefficients for temperature and richness remained close to 0 across England and Wales (Figure 4.5c). They began most negative in the south-east of England, and most positive in north-Wales and central southern England, becoming most negative in the east of England (Figure 4.5c). Slope coefficients for temperature and CA1 score were close to 0 in the first half of the time series, whereas by the second half the west of England and the whole of Wales were negative (Figure 4.5d). Relationships between pH and richness were mostly neutral through time; coefficients became more negative in north Wales, the north-western coast and the south-west of England (Figure 4.6a). Relationships between pH and CA1 score were close to neutral but mostly negative; they began most negative in the south-east, before weakening by 2005 – 2019 (Figure 4.6b).

Arable land and improved grassland had a mostly positive or no relationship with richness at the start of the time series, with the exception of the east of England for arable land, and areas of the south and northern England where this was slightly negative (Figure 4.6c and 12a). Coefficient gradients were less positive by 2005 – 2019 for both land use types, with more negative coefficients in the east of England for arable land. Arable land and CA1 score

had mostly negative relationships across England and Wales through time; in 1991 – 2005 there was a small area with a positive relationship in south-west England and this expanded to include some areas in the east of England in 2005 – 2019 (Figure 4.6d). Relationships between improved grassland and CA1 score were consistently mostly neutral across the UK (Figure 4.7b). The relationship between urbanisation and both invertebrate measures was mostly negative (Figure 4.7c and d) and coefficients remained relatively consistent through time. In the first half of the time series, there were a few locations where the relationship between urbanisation and richness was slightly positive around the east of England and the East Midlands, which was absent by 2005 – 2019 (Figure 4.7c).



Figure 4.4 Maps of regression coefficients calculated from GWR models for phosphate concentration for (a) richness and (b) CA1 score and nitrate concentration for (c) richness and (d) CA1 score. Positive values (blue) indicate a positive correlation between invertebrate measures and environmental variables, while negative values (orange) indicate a negative correlation.



Figure 4.5 Maps of regression coefficients calculated from GWR models for biological oxygen demand for (a) richness and (b) CA1 score L00 and water temperature for (c) richness and (d) CA1 score. Positive values (blue) indicate a positive correlation between invertebrate measures and environmental variables, while negative values (orange) indicate a negative correlation.


Figure 4.6 Maps of regression coefficients calculated from GWR models for pH for (a) richness and (b) CA1 score and arable land for (c) richness and (d) CA1 score. Positive values (blue) indicate a positive correlation between invertebrate measures and environmental variables, while negative values (orange) indicate a negative correlation.



Figure 4.7 Maps of regression coefficients calculated from GWR models for improved grassland for (a) richness and (b) CA1 score and urban land for (c) richness and (d) CA1 score. Positive values (blue) indicate a positive correlation between invertebrate measures and environmental variables, while negative values (orange) indicate a negative correlation.

#### 4.4.3 Structural equation models

Structural equation models identified complex relationships between environmental and biological variables that were largely consistent with the relationships hypothesised (Figure 4.8 and 4.9). Fewer direct relationships were identified between invertebrate family richness and water quality variables, land use and hydrology, compared to CA1 score. Notably, nitrate had no significant pathways with richness or any other water quality variables and so was absent from the richness SEM.

#### 4.4.3.1 Richness

pH (positive relationship), phosphate and BOD (both negative) were the only water quality variables to have direct relationships with richness. Discharge had a negative relationship with richness, although this was at a lower significance level (Figure 4.8). Amongst land cover variables, urbanisation had a large negative direct impact on richness (Figure 4.8), whilst improved grassland had a weaker, positive effect, and there was no direct relationship with arable land cover. All three land cover classes had indirect effects upon richness via 3–5 direct effects on water quality and climate variables (Figure 4.8). Arable land cover had the fewest paths but the strongest direct effect on another variable, with a large positive path with discharge. Arable land also had negative paths with BOD and temperature. Urbanisation had paths to every water quality variable and discharge, with a negative relationship with discharge and positive paths to pH, phosphate, temperature, and BOD (Figure 4.8). This indicated the multi-faceted nature of urbanisation effects on richness via its effects on water quality and discharge. Improved grassland had direct pathways to all water quality variables, except for water temperature. While urbanisation had a positive pathway with phosphate, this was negative for improved grassland. Discharge had significant negative pathways with phosphate, BOD, and temperature. Temperature and phosphate were the only water quality variables to have direct pathways with another water quality variable (Figure 4.8). Temperature having a negative pathway to BOD while phosphate had a positive pathway to BOD.

## 4.4.3.2 CA1 score

Significant direct pathways were found between all water quality variables, land use and discharge with CA1 score, except for temperature, which indirectly impacted CA1 through a negative pathway with BOD (Figure 4.9). Urban land cover and discharge had the strongest

direct pathways with CA1 score (Figure 4.9), the former similar to the richness SEM (Figure 4.8). Nitrate, pH, phosphate, BOD, and improved grassland had negative direct pathways to CA1 score. The strongest pathway within the SEM was from arable land cover to discharge, resembling the richness SEM. Arable land cover negatively influenced BOD and temperature, but to a lesser degree. Urbanisation had direct paths with each water quality variable, and discharge. This was negatively associated with just discharge, while having positive pathways with nitrate, pH, phosphate, BOD and temperature. Improved grassland, as with the richness SEM, had pathways to all the same variables as urbanisation, except for temperature where there was no significant path. Improved grassland had a negative pathway to nitrate, pH, phosphate and discharge, while having a positive pathway to BOD. Discharge had positive associations with nitrate, while having negative pathways to phosphate and BOD. Similar to the richness SEM (Figure 4.8), temperature and phosphate had pathways with BOD which were negative and positive, respectively.



Figure 4.8 Structural equation model explaining water quality and land use effects on national scale river invertebrate richness. AIC 108, Fisher's C = 12.38 with *p*-value = 0.42 and on 12 degrees of freedom. Orange arrows indicate negative relationships, green arrows represent positive relationships. Thickness of arrows represent the magnitude of the standardised regression coefficient, with exact values noted alongside each arrow. Each line indicates significant paths, \*, \*\* and \*\*\* representing *p*-values of  $\leq$  0.05,  $\leq$  0.01, and  $\leq$  0.001, respectively.



Figure 4.9 Structural equation model explaining water quality and land use effects on national scale river invertebrate CA1 score. AIC 121, Fisher's C = 11.21 with *p*-value = 0.34 and on 10 degrees of freedom. Thickness of arrows (dashed, solid, bolded) represent the magnitude of the standardised regression coefficient, with exact values noted alongside each arrow. Each line indicates significant paths, \*, \*\* and \*\*\* representing *p*-values of  $\leq$  0.05,  $\leq$  0.01, and  $\leq$  0.001, respectively

## 4.5 Discussion

Investigating changes in invertebrate communities and identifying drivers is complex, especially at large spatial scales. While quantifying long-term trends with single indices can provide evidence for how communities are changing, expanding on this to investigate trends across spatial scales can add weight to evidence. Natural and anthropogenically-driven environmental conditions are known to vary across spatial scales, which will influence communities differently across space and time (Davies and Neal 2007; Rothwell *et al.* 2010a). As interactions between environmental conditions can lead to unpredictable influences on communities, some drivers of invertebrate communities can be overlooked when investigating individual environmental variables in isolation (Lefcheck 2016). By using novel approaches and multiple methods, it was hoped that the current analysis would provide new insights into drivers of communities and address some of the challenges in approaching research questions at large scales. This type of analysis was made possible by the extensive monitoring of riverine systems in England and Wales. This enabled questions to be addressed at scales relevant to land managers and government, to inform policies or management actions.

This study revealed the extent to which temporal changes in macroinvertebrate communities varied across England and Wales, providing a more nuanced story than overall indices of change (Chapter 3). Decline in richness and CA1 score were found in fewer locations than increases, consistent with previous work which has found overall increases in richness and CA1 score within this time period in England and Wales and Hypothesis 1 (Vaughan and Ormerod, 2012; Pharaoh, Ormerod and Vaughan, 2021). Locations where richness and CA1 score were declining were positioned, for the most part, in areas where these measures were highest in 1991 – 1993. Indicating that declines have occurred in the most diverse sites, with the exception of richness in north-east England. Prior work has suggested that improvements in the most degraded urban areas is leading to these "closing the gap" on sites of better water quality, which has been used to explain increasing homogenisation through this time found in Chapter 3. Evidence of decline amongst these highest quality sites does, however, indicate that while urban areas are improving at a rate greater than other sites, declines may be partly contributing to homogenisation through time.

Results echo prior analysis by Vaughan and Ormerod (2012), although there appear to be some areas where the direction of change through time differs with the additional 11 years of data. For example, richness and CA1 increased in parts of north-east England from 1991 to 2008, whereas in this analysis a decline was observed between 1991-3 and 2017-19. CA1 score in the south-west of England and a small area in the north-west of England also changed from an initially neutral/negative change by 2004-2005 but increased slightly by 2017 - 2019. This indicates that in some areas the trajectory of change varied over time, perhaps due to changes in land management or water quality over the years 2009-2019. Declines in richness were observed in some areas of lowland and south-west England and Wales, consistent with Vaughan and Ormerod (2012). These areas are dominated by improved grassland and had the highest taxonomic richness at the start of the time series. The relationship between improved grassland and richness in the GWR became less positive through time. Potentially indicating that changes in practices could be behind this reduction in richness, or perhaps driven by a water quality or hydro-geomorphological variable not included in these analyses. For example, coliform bacteria in rural rivers has been positively linked to improved grassland cover (Crooks et al. 2021), suggesting that livestock pharmaceuticals could be entering rivers in the same way as animal faecal matter. This has the potential to impact water quality and invertebrate richness, but is absent from these analysis.

There was evidence of losses of pollution-sensitive taxa (reduced CA1) across much of the English and Welsh uplands, yet there was little decline in richness in these areas, partially supporting Hypothesis 1. This suggests that turnover has occurred in these areas, with more tolerant species replacing these pollution-sensitive taxa. These results highlight the importance of considering multiple facets of diversity, as this detail would be missed if relying on richness alone. Uplands have had historic acidification pressures across England and Wales. Acidification has been ameliorated through legislative actions and industrial decline in Europe, leading to reduced emissions and deposition, and increases in pH (Ormerod *et al.* 2010; Broadmeadow *et al.* 2019; Whelan *et al.* 2022). Despite this, ecological recovery in acidified streams has been considered delayed and incomplete (Murphy *et al.* 2014). This is due in part to episodic low pH, limitations to dispersal and the resistance of low pH tolerant communities to recolonisation by circumneutral species

(Kowalik *et al.* 2007; Ormerod and Durance 2009; Frame *et al.* 2016; Gray *et al.* 2016). Uplands in the UK have also been historically subject to coniferous afforestation, which has had lasting impacts on the hydrology, water chemistry and ecology of upland streams (Bunce *et al.* 2014). Upland streams and their communities are also likely to be more sensitive to climate change (Macadam, England, and Richard, 2022). Potential sensitivity is due to their fast response times to hydrological events (Gomi *et al.* 2002; Caissie 2006), as well as their higher elevation meaning species are limited in their potential to move to cooler areas at higher altitudes. Climate change can also have secondary effects on upland stream conditions (e.g. organic litter dynamics), which can further influence invertebrate communities (Pye *et al.* 2022). Losses of pollution-sensitive taxa from within these areas could be due to unstable communities, which have yet to recover from acidification, being further impacted by other environmental pressures such as climate change. This is further supported by the strengthening negative correlation through time with temperature in the north and west seen in this study, which may be indicative of a climate signal.

Consistent with Hypothesis 2, it is apparent that the impact of water quality variables and land use on invertebrate community measures vary markedly across England and Wales. Spatial variation has been considered an explanation for difficulties in determining drivers of invertebrate communities in past research (Vaughan and Ormerod, 2012; Pharaoh, Ormerod and Vaughan, 2021), with GWR results confirming this for each water quality variable. Correlations between environmental variables and richness and CA1 score across England and Wales do not appear to be consistent, suggesting that these potential drivers are impacting richness and CA1 score differently. Relationships did not just vary across space, but also through time for some water quality determinands, contradicting Hypothesis 2. For example, the relationship between nitrate concentration and invertebrate richness in south Wales changed from negative to positive. This may be driven by changes in the concentrations of water quality variables in some instances leading to changes to primary production and altering physical habitats (e.g. increases in algal abundances), although in this case nitrate concentration had remained relatively static. The changing mix of multiple stressors, not all of which were included within this analysis, has the potential to change the apparent relationships between focal variables and invertebrates. For example, contamination with heavy metals or habitat alteration such as dredging has the potential to

override the effects of warming on freshwater ecosystems (Morris *et al.* 2022). This alludes towards complex relationships between water quality variables and invertebrate measures, which are overlooked in this type of exploratory analysis. This is partly because many key pressures are not routinely measured, or have not been monitored in the past (e.g. pesticides, pharmaceuticals). This means that many key pressures are not possible to include in these and other analysis (Whelan *et al.* 2022). Additionally, biotic interactions are not captured within these analysis, which may also affect community-level responses to stressors (Larsen and Ormerod 2014).

Arable land had the most varied coefficients across space for CA1 score and richness, having the strongest negative and positive relationships. Arable land management practices and their impact on waterbodies can vary greatly according to the crop in production (Williams et al. 2010; Boardman et al. 2020). Across the UK, certain areas are dominated by different types of arable agriculture, for example west-Wales is dominated by potato crops, while the greatest density of sugar beet is located in the east of England (England Agricultural Census, 2016; Wales Agricultural Census, 2018). The locations of arable agriculture, for example arable agriculture in locations with high risks of soil erosion (e.g. sloped areas) can increase sediment delivery to watercourses (Walling et al. 2002; Walling et al. 2006; Rickson 2014). These differences are likely to influence water quality, impacting invertebrate communities differently, which will likely be reflected in these trends. This represents a limitation of land cover data which overlooks subtleties in specific agriculture types. Land cover data used in the analysis was taken from the mid-point of the timeframe, as these data were not suitable for comparative analysis between years, while water quality data spanned the timeframe. As there has been some land cover change during this timeframe (Rowland et al. 2020), this is a limitation to the analysis.

On the whole, relationships between water chemistry, land use and temperature with invertebrate measures varied through time and between each measure. It could be expected that these relationships would be negatively associated with concentrations of nitrate and phosphate, with the most negative correlation coefficients where nutrient concentration was highest (worst water quality) (Rico *et al.* 2016; Nessel *et al.* 2021). A more positive coefficient at lower nutrient concentrations could also be expected, where nutrient concentration could be a limiting factor to primary production leading to an

indirect link with macroinvertebrate communities (Kiffney and Richardson 2001). This hypothesis would, however, require the use of absolute nitrogen and phosphorous concentrations and confirmation that concentrations are above limiting levels. While a positive coefficient is found in some areas with lower nutrient concentrations for some variables, it is not a consistent theme. This suggests that relationships are not clearly governed by the concentrations of either water quality variables or the extent of land cover.

This study provides evidence that some water quality variables, land use and discharge are influencing river invertebrate communities, and reveals relationships amongst these variables. For example, a large positive relationship was found between arable land and discharge in both models. This could be driven by increased runoff from arable land, due to periods without vegetation cover following harvest, field drainage or compaction of soil by vehicles (O'Connell et al. 2007). Additionally, arable land may be associated with larger rivers which have higher discharge, as arable land is typically located within the lowlands which have larger catchments. Drivers of richness and community composition, while similar, were not identical in terms of variables included, direction of relationships (positive/negative) and the magnitudes of relationships. The directions of most relationships shared between the richness and CA1 score models were identical. The exception was pH and improved grassland, which had positive relationships with richness and negative relationships with CA1 score, and discharge, which had a positive relationship with CA1 score and was negative related to richness. Differences would be expected, however, since different aspects of community diversity are known to vary in their response to environmental variables (Vaughan and Ormerod, 2012; Grimaldo et al. 2016). Whilst CA1 score and richness have shown generally improving trends in England and Wales within this timeframe (Vaughan and Ormerod, 2012; Pharaoh, Ormerod and Vaughan, 2021), it is important that these are considered as independent entities. This is evidenced by the contrasting spatial patterns of CA1 score and richness changes through time, alongside varying spatial relationships with environmental variables. Despite this, urbanisation, BOD and phosphate were found to have consistently negative relationships with both richness and CA1 score.

Clear links were identified between land cover and water quality from the SEM, suggesting that these factors are partially responsible for the resulting river water quality. Urbanisation

appeared to be the greatest shared direct driver of richness and CA1 score. This suggests that variables not included within the model, but connected to urbanisation, are responsible for invertebrate community changes. Urban rivers are subjected to a host of artificial changes, such flow alteration and bank modification which can influence communities, in addition to changes in water quality (Walsh et al. 2005; Miller and Hess 2017; Zerega et al. 2021). Urban streams are also exposed to a host of other stressors, from stormwater overflows, sewage treatment work and industrial activity outflows, and surface runoff. This can include persistent contaminants, personal care products, and microplastics, which can influence and interact with invertebrate communities and contribute to a complex response (Vane et al. 2010; Tibbetts et al. 2018; Windsor et al. 2019a). Discharge had a similarly large impact on CA1 score, while also impacting other water quality variables. This could indicate that discharge underpins changes in invertebrate communities via other water quality variables that were not included in this analysis. The environmental and water quality variables in this analysis were not exhaustive. However, the complex relationships evidence in this analysis capture some of the key drivers and their interconnections impacting invertebrate communities over space and time.

## 4.6 Conclusion

Temporal changes in invertebrate communities varied across England and Wales, with national trends that are consistent with overall biological recovery (Chapter 3) disguising localised declines. The use of GWR in this study highlights how relationships between water quality, climatic variables and land use with invertebrate communities vary across space, illuminating the challenges of pinpointing the drivers of community trends at national scales. This furthers analysis in Chapter 3 and other analyses, which have found that trends through time vary across land use types. This leads to questions of why these relationships vary across space and time, and what variables may be responsible for this. Structural equation modelling in conjunction with GWR demonstrates the complexity of interconnected freshwater systems. SEM is able to demonstrate how environmental variables interact, and explain why relationships between these and invertebrate measures can be difficult to unpick. The interconnected relationships between variables begin to suggest some reasons for results from GWR analysis. As environmental measures were found to influence one another, these variables may partly explain why relationships between abiotic and biotic factors vary across space and time. While national scale studies provide invaluable insights into trends in communities required by policy makers and land managers, these results demonstrate the limitations that studies of this scale face in attributing environmental variables to such trends. Catchment-scale research will therefore play the most valuable role in providing evidence for the drivers of trends, which are needed to govern important management decisions.

# Chapter 5: Invertebrates in the river Wye – A case study investigating community trends, the role of land use and environmental variables

# 5.1 Summary

Macroinvertebrate communities have changed substantially across England and Wales over the last 30 years, with evidence of ongoing recovery from historical sanitary pollution in particular (Chapter 3). The national picture may disguise regions of degradation or faster recovery (Chapter 4), and it may be difficult to uncover complex relationships between communities and the environment at coarse spatial scales. Individual catchments can exemplify many of the challenges facing rivers at national scales, whilst being more amenable to the use of high-resolution data and working at management-relevant scales. The river Wye captures many of the issues facing Europe's rivers, from historical problems with acid deposition in its upper reaches, through to the expansion and intensification of agriculture in the mid- and lower-reaches, alongside localised urbanisation. Using macroinvertebrate data collected from across the Wye catchment (1991-2019), allied to water quality, climate and land cover change data, this chapter aims to: (i) quantify changes in invertebrate communities over 29 years, including overall richness, community composition and functional diversity, (ii) identify how community composition has changed, and identify its relationships with catchment characteristics, and (iii) investigate trends in traits and identify how these relate to land use. Taxonomic and functional richness increased through time, while little change in taxonomic and functional composition occurred. Changes in water quality were not found to be related to temporal changes in any invertebrate measure. The extent of arable land in the catchment explained the largest proportion of variation in community composition, followed by altitude and grassland. Despite this, the magnitude of catchment land use change (grassland and arable) between 1990 and 2015 was not correlated with the extent of change in community composition. These results show that while using individual catchments to investigate biodiversity change in rivers can enable more detailed analysis, identifying links between environmental variables (particularly water quality variables) is challenging. This is likely due to the interconnected nature of variables, as identified in Chapter 4. Of particular relevance to the

Wye, this chapter found no dramatic decline in the biological quality. This is despite recent land use changes which are thought to have caused a decline in the condition of the river.

## 5.2 Introduction

The disproportionate threats to freshwater systems and their declining condition warrants wide ranging research into their status, ecology and responses to the array of stressors they are exposed to (Dudgeon *et al.* 2006; Vörösmarty *et al.* 2010; Reid *et al.* 2019). Large national or international studies provide essential insight into overall status and trends of freshwaters, and reveal broad patterns in the relationships between catchment variables with river condition and biota (e.g. Chapter 3; Pilotto *et al.* 2020; Haase *et al.* 2023; Rumschlag *et al.* 2023). This is important for providing evidence at a scale which can support policy and management decisions.

Whilst broad scale studies have a great potential to derive generalisations, there are, however, limitations to working across large spatial scales. For example, relationships and trends which vary across space can cancel out one another when combined, disguising regional and local scale variation (e.g. Chapter 4; Levin, 1992). Alternatively, drivers not included within the study design but which vary across large scales can affect results (Smart *et al.* 2012). Moreover, it is possible to collect and analyse more detailed or higher resolution data sets at smaller scales, offering new insight. Studies at smaller scales tend to be affected by less background heterogeneity, potentially making it easier to identify relationships (van Rensburg *et al.* 2002; Stein *et al.* 2014). For instance, interspecific interactions in some cases can be observed at small scales, but not broader scales (Sherry and Holmes 1988; Levin 1992). Finer scale studies also have a greater potential for hypothesis testing, are more replicable and generally have a higher resolution of detail (Wiens 1989).

The catchment is a natural scale at which to work on rivers, as activities within river catchments are known to be reflected in the river itself (Hynes 1975; Frissell *et al.* 1986; Allan and Johnson 1997). This has made the catchment a standard scale for many river management decisions and activities to take place (Mohamad Ibrahim *et al.* 2019; Klaar *et al.* 2020; NRW EA and Natural England 2021). As such, there is a long history of catchment-based studies (Casey *et al.* 1993; Lange *et al.* 2014; Tye *et al.* 2016). With careful catchment selection, many of the issues of concern across national scales can be exemplified, whilst

retaining the benefits of working at smaller scales. In the UK, the river Wye is such a catchment because it contains a range of different land uses, altitudes and is relatively well studied (see methods). Across England and Wales, there have been large scale changes in invertebrate communities (Chapter 3), underlain by marked regional variation (Chapter 4), with particular concerns around water quality changes. These often relate to land use, with the sources of many pressures attributed to agricultural activities (e.g. intensive agriculture) and urbanisation (e.g. runoff, effluent discharge). The impacts of these activities on the physical conditions of rivers and their biodiversity varies widely, according to land use type and intensity.

The river Wye exemplifies many of the issues affecting rural rivers in particular, especially nutrient and fine sediment pollution caused by the agricultural activities dominating the catchments of rural rivers (Chapter 2; Davis et al. 2018). A Special Area of Conservation (SAC) and well-studied catchment providing an array of background information, its status has been a source of controversy in recent years. This has been brought into the public consciousness through coverage by media outlets, concerned groups and environmental organisations (Robin McKie 2020; Rivercide. 2021; Morfett 2022; Blakely 2023; River Action UK 2023). Ecologically, the Wye has seen a decline in salmon (Environment Agency 2021b) and evidence of eutrophication impacts on macrophyte communities, while invertebrate communities are cited as generally healthy (Environment Agency 2022b). Significant algal blooms are also recorded in the summer, attributed to excessively high summer temperatures and phosphate pollution (Environment Agency 2021b; Environment Agency 2022b). The available evidence regarding change is, however, largely derived from a mix of water chemistry data and anecdotal accounts, resulting in an incomplete picture of overall change. Chapter 4 hints at increases in invertebrate richness within the river Wye catchment, with a shift towards more pollution tolerant taxa in the upper Wye and a shift towards pollution sensitive taxa in the lower Wye. This implies that invertebrate trends are likely to be mixed – potentially driven by different land use activities.

The Environment Agency (2022) largely attribute the declining status of the Wye to orthophosphate additions in the river from arable agriculture. Discharge from sewage treatment works (STWs) are also listed as significant contributing factors to orthophosphate concentrations. In a recent River Wye Core Management Plan (NRW 2022), NRW cite

agriculture and forestry operations as the main sources of diffuse pollution and siltation in the Wye. In the River Wye SAC Nutrient Management Plan, agricultural activities are credited with a large portion of the phosphate load, followed by STWs (NRW, EA and Natural England, 2021). The Wye was also found to be at a particularly high risk of pollution from the land due to the soils' poor ability to hold applied phosphorous – which have been applied in a surplus – combined with moderate to high rainfall (Withers *et al.* 2022).

With the impacts of different land use types and intensities known to reflect differently on the water quality and ecological status of rivers (Weijters *et al.* 2009; Clapcott *et al.* 2012; Crooks *et al.* 2021), changes in land use through time would be expected to affect the condition of rivers (Bussi *et al.* 2018; Wijesiri *et al.* 2018). Within the Wye, land use has changed in recent years, including a shift from pastoral land towards a greater coverage of arable agriculture in the mid- and lower-catchment (Rowland *et al.* 2020; Environment Agency 2022b). In addition, and a source of contention, is the role that increasing intensive poultry units may play in declining river quality within the Wye, due to the spreading of manure on land across the catchment (Hatton-Ellis and Jones 2021; Morfett 2022; NRW 2022).

Using data from the years 1991–2019, covering 113 locations from across the Wye catchment, this study aimed to quantify changes in invertebrate communities through time and identify drivers of change. This builds on the work of Chapters 3 and 4, with a focus on land cover change and expanding the use of biological traits. Assessment of changes in biological traits through time is considered a powerful method to evaluate the condition of an ecosystem, especially when combining functional and trait measures with taxonomic measures (Mouillot *et al.* 2013). Whilst there is some evidence of improvements in the condition of agricultural catchments nationally (Chapter 3), agricultural intensification is expected to lead to declining biological condition within water bodies, including losses of sensitive taxa (e.g. Kibichii *et al.* 2015) and some traits (e.g. Larsen and Ormerod, 2010).

Specific aims of this study were to: (i) quantify changes in invertebrate communities at the whole-catchment scale over 29 years, (ii) evaluate the extent to which changes in water quality, temperature and discharge accounted for catchment-scale trends, (iii) investigate changes in the prevalence of different biological traits in catchments with different land

cover, and (iv) look in more detail at how assemblages have changed between the start and the end of the time series, and in response to changing land cover. It was hypothesised that:

- Hypothesis 1: Taxonomic and functional richness will increase throughout the time series, whereas there will be little change in the average composition and prevalence of different biological traits – due to contrasting changes in the lower and upper Wye catchment observed at the coarse national scale (Chapter 4) cancelling one another out.
- Hypothesis 2: Variation in the macroinvertebrate communities will mostly be accounted for by arable and improved grassland cover within the catchment. Phosphate will be the greatest water quality driver of trends.
- Hypothesis 3: Sampling site altitude, and the extent of arable and improved grassland cover in the catchment, will account for the largest share of variation in the invertebrate community.
- Hypothesis 4: Some community weighted mean (CWM) traits will have changed through time, with declines in large-bodied and longer-lived taxa which may be more sensitive to agricultural intensification (Larsen and Ormerod 2010). Community weighted mean traits will vary with arable and improved grassland percentage cover; these relationships will differ for both land use types – reflected by their different impacts on environmental conditions.
- Hypothesis 5: Site dissimilarity from the start to the end of the time series will be greater where larger changes in land cover have occurred.
- Hypothesis 6: Beta-diversity will decline from the start to the end of the time series due to the anticipated losses and gains of pollution-sensitive taxa in the upper- and lower-Wye respectively (Hypothesis 1).

# 5.3 Methods

## 5.3.1 Study area

The River Wye is the fourth longest river in the UK, with its source in the Cambrian Mountains in mid-Wales, from where it travels 250 km to the Severn Estuary near the town of Chepstow. Its catchment area is 4,131 km<sup>2</sup>, spanning the border between England and Wales. The Wye catchment is largely rural, with the small city of Hereford with a population of 187,100 (ONS 2021) making up the greatest density of urban land cover within the catchment, with smaller population centres in Chepstow, Leominster, Ross-on-Wye, Llandridod Wells and Monmouth (Figure 5.1). The Wye catchment contains major tributaries, including the Ifon, Ithon, Lugg, Arrow, Frome, Monnow and Trothy (Figure 5.1). The catchment is predominantly agricultural, with primarily arable and mixed farming (cereals, potatoes, hops, soft fruit, dairy and poultry) in the more fertile, productive lowlands. Pastoral farming dominates the north of the catchment, including more intensive improved grassland and less intensive rough and acid grassland in upland areas (Figure 5.2a and b). An increasing proportion of the catchment land has been managed for arable agriculture over the years 1990-2015 (Rowland et al. 2020), and since 2016, especially for the cultivation of maize and potato - which can lead to soil loss (Environment Agency 2022b). Several conservation designations cover parts of the catchment: the river Wye and Lugg (its main tributary) are designated Sites of Specific Scientific Interest (SSSIs), the main Wye is a Special Area of Conservation (SAC) along its entire length, and 326 km<sup>2</sup> of the lower catchment form the Wye Valley Area of Outstanding Natural Beauty (Wye Valley AONB Office 2021).



Figure 5.1 Map of the Wye catchment showing major settlements and tributaries from OS Open Rivers (1:25 000).



Figure 5.2 a) Arable, grassland (improved, rough and acid) and suburban land use in the Wye catchment, based on the CEH land cover map (Morton *et al.* 2014), b) Altitude within the Wye catchment ≥250 m.

The river Wye and its catchment have been widely covered in the scientific literature, across a range of fields. This has ranged from ecological research, including surveying invertebrate communities (Brooker and Morris 1980), trout (Crisp and Beaumont 1996), species interactions (Jenkins and Ormerod 1996), animal behaviour studies (Yoerg 1998) and investigating the impacts of terrestrial activities on river ecology (Gee and Smith 1997). Work has also investigated water quality and hydrology in the Wye, including modelling the effects of future climate and land use change projections on hydrochemistry and ecology (Bussi *et al.* 2018), linking water chemistry to factors such as geology, land use and population (Oborne *et al.* 1980) and in-depth study of decadienal nutrient fluxes and patterns in nutrient water quality (Jarvie *et al.* 2003).

### 5.3.2 Data preparation

The same starting data set was used as in Chapters 3 and 4. Environment Agency and Natural Resources Wales macroinvertebrate data covering 29 years (1991–2019 inclusive), were trimmed to include only sites located within the river Wye catchment (see Chapter 3 for details of data selection and processing). This left a total of 133 macroinvertebrate biology sites, representing 978 samples (mean = 8.7 visits per site; Figure 5.3a). Taxon abundances were recorded as either raw counts or placed in log<sub>10</sub> abundance classes (e.g. 1-9 and 10-99 individuals). To use abundance, all data were harmonised into log<sub>10</sub> classes and converted to the midpoint for each class (e.g. the abundance for taxa in the 1–9 class was recorded as 5).



Figure 5.3 Location of River Wye catchment within England and Wales. (a) Blue points are locations of the 113 macroinvertebrate biology sites within the Wye catchment. (b) Orange points are locations of 64 macroinvertebrate sites which could be paired to water quality sites.

The first axis (CA1) from a correspondence analysis, which was run for sites within the Wye using R's vegan package (Oksanen *et al.* 2019), was used to summarise the major variation in community composition. Analysis was run using presence-absence data (eigenvalue = 0.228). Similar to analysis at the national scale (Chapters 3 and 4), negative CA1 scores represented communities with greater proportions of taxa associated with poorer water quality, slower flow and siltier substrata (e.g. Oligochaeta and Mollusca). Positive scores were indicative of taxa typical of better water quality, faster flows and greater oxygenation (e.g. Ephemeroptera, Plecoptera and Trichoptera). See Appendix D for the taxon loadings (Appendix D Table D.1). Correspondence analysis loadings for composite and family groups (Appendix D Table D.1) were positively correlated with Biological Monitoring Working Party (BMWP) taxon scores (Hawkes 1998), confirming that increasing CA1 scores reflected the increasing prevalence of pollution sensitive taxa (Spearman rho = 0.42, p = <0.001).

Functional richness was calculated using seven traits described by Tachet et al. (2010) that focused on food and habitat preferences: maximum size, feeding habit, food, locomotion and relation to substrate, preferred current velocity and preferred substratum (Appendix D Table D.2). Genus-level data on traits were downloaded from freshwaterecology.info (Schmidt-Kloiber and Hering 2015) in the form of affinities (weights) for the subcategories within each of the seven traits. These were converted to family-level traits by calculating each family's mean affinity for each subcategory from its constituent genera, weighting each genus by its relative abundance across the 2505 samples that formed the original RIVPACS calibration data for the UK (Wright et al. 2000; Monaghan and Soares 2012). Affinities were then re-scaled so that the sum of the subcategories within each trait summed to one for each family (Vaughan and Ormerod, 2014). This was possible for 69 of the families which formed this part of the analysis (Appendix D Table D.1). Functional richness was calculated using the dbFD function in the R package FD (Laliberté et al. 2015), selecting four principal coordinate analysis (PCoA) axes using the methodology of Maire et al. (2015): 0.1 % of samples had to be removed where the number of taxa present was less than the number of PCoA axes.

Changes in average trait composition were described using community weighted means (CWMs) and fuzzy correspondence analysis (FCA): both were calculated using the same set of traits used to calculate functional richness. Community weighted means were calculated

using the dbFD function, based on the presence-absence data (i.e. families had equal weightings). Fuzzy correspondence analysis was performed using the dudi.fca function in ade4 (Dray and Dufour 2007), taking the first axis from the analysis to provide a summary of the major variation in functional composition from RIVPACS weighted traits. This summarised the main changes, equivalent to (taxonomic) CA1 above. Only the first axis (FCA1) was retained, explaining 31.31 % of variation. Traits used for calculation of functional richness, CWMs and FCA1 are listed in Appendix D (Table D.2). Positive FCA1 scores represented organisms with a greater affinity for faster current velocities and coarser substrata. Positives scores represent a greater prevalence of scrapers, shredders and deposit feeders, and organisms which feed on detritus, fine sediment, and microorganisms. Negative FCA1 scores represent organisms with preferences for finer substrates (e.g. silt and organic detritus), and slower current velocities, and greater prevalence of more active organisms which are predatory and parasitic.

The catchment was delineated for each macroinvertebrate site (see Chapter 3) and used to calculate the percentage of land use and land cover for each site. Two types of data were collected, as both lend themselves to different analysis within this thesis. Firstly, the percentage of urban or suburban landcover, improved grassland or arable agriculture were calculated from the more detailed 1 km resolution UK-CEH Land Cover Map 2007 (Morton *et al.* 2014) – the landcover map closest to the centre of the time series. Secondly, land cover was extracted for 1990 and 2015 from the 25 m raster Land Cover Change 1990-2015 map (Rowland *et al.* 2020), which uses six simplified classes, with grassland, arable and built-up areas extracted. This simplified map is suitable for comparing changes through time, unlike the standard UK-CEH land cover maps which are not suitable to measure changes through time due to differences in methodology. Therefore land cover detail or temporal detail is traded, meaning that using each of these types of data are used for different purposes within this chapter. Catchment variables: calcareous geology, mean annual precipitation, specific stream power, base flow index (BFI), and stream power were gathered using methodology from Chapter 3.

Further filtering of the data set was used to create a second data set for linking the macroinvertebrate community to water quality and discharge. The same water quality and discharge data were used as in Chapter 4, utilising the calculated annual median water

quality and site discharge controlled for catchment size. Due to a more limited number of sites within the Wye, the compromise of data detail for the quantity of sites to be retained was vital to enable this analysis. Water quality and gauging data were trimmed to include sites within the Wye catchment (see Chapter 3 for details of data selection and processing). Macroinvertebrate sites were paired with water chemistry and discharge sites in close proximity, using maximum distances of 1 km to the nearest water chemistry location and 7 km to a gauging station (Vaughan and Ormerod, 2012). Data were filtered at each site so that only years with macroinvertebrate, chemistry and discharge data were used. This resulted in a data set of 64 macroinvertebrate locations, with a mean of 3 years sampled at each site (Figure 5.3).

#### 5.3.3 Temporal patterns in invertebrate communities

To test Hypothesis 1, catchment-wide temporal trends in macroinvertebrate taxonomic and functional richness, and CA1 and FCA1, were estimated for the time period 1991–2019 using generalised additive models (GAMs), fitted using R's mgcv package (Wood, 2011). The approach followed Vaughan and Ormerod (2012, 2014), using Fewster *et al.*'s (2000) methodology for estimating smoothed trends. The four metrics were modelled as a function of year using cubic regression splines. The models also included the 12 site and catchment level environmental covariates to account for variation in macroinvertebrate assemblages between sites (Fewster *et al.* 2000; Vaughan and Ormerod, 2012). The degrees of freedom (smoothness) of the year term was fixed at nine, approximately 1/3 of the number of years, as this is a good compromise between identifying long-term trends and shorter-term (multiple-year) changes (Fewster *et al.* 2000). Bootstrapping was used to produce nonparametric 95 % confidence limits of the trends, based on 399 bootstraps. Significant positive and negative 'change points' (inflections) in the trend were identified using bootstraps of the trends, following Fewster *et al.* (2000).

## 5.3.4 The role of water quality and discharge in macroinvertebrate trends

To test Hypothesis 2 and 3, generalised additive mixed-effects models (GAMMs) were used to investigate the extent to which temporal variation in the macroinvertebrate communities could be accounted for by changes in water quality, temperature and discharge. After controlling for sampling site location and the amount of arable and improved grassland land cover in the catchment (urban land use was excluded from this analysis, as just two sites had >1 % urbanised land cover, 2 % and 14 % respectively). Water quality and discharge variables were centred for each sampling site by subtracting the mean values at those sites, leaving deviates which had a mean of zero for each site and focused on temporal change (cf. among-site differences). Four GAMMs – for CA1, richness, FCA1 and functional richness – were fitted using the mgcv package in R (Wood, 2017). All covariates were fitted using regression splines, with the degree of smoothing chosen using the default generalised cross-validation process (Wood 2017), allowing nonlinear relationships to be modelled where appropriate. The following variables were included in the models: i) site-level covariates: proportion of catchment with improved grassland and arable land; ii) site location: OS National Grid eastings and northings, modelled as a two-dimensional tensor product smooth; and iii) time-varying covariates: deviates calculated from median BOD, pH, nitrate, phosphate, water temperature and discharge. Sampling site was included as a random term in the model to account for repeat sampling through time, and a first-order autoregressive function used to model residual temporal autocorrelation (Pinheiro and Bates, 2000).

#### 5.3.5 Changes in community structure in response to changing land cover

To test whether the invertebrate community changed in response to changing landcover, data were extracted for the first and last four years (1991-1995 and 2015-2019) and linked to the land cover change dataset. Abundance data at each site were averaged within each of these timepoints if a site was sampled more than once, and sites were only retained where they appeared in both these timeframes (n = 71 sites). A total of 66 invertebrate families were present in this remaining dataset (Appendix D Table D.1). Non-metric multidimensional scaling (NMDS) was undertaken based on Bray-Curtis dissimilarities for the natural logarithm of invertebrate family abundance. This used metaMDS in the vegan package (Oksanen *et al.* 2020) with a two-axis solution (stress = 0.16).

To test the hypothesis that invertebrate beta-diversity decreased through time (Hypothesis 6), differences in the beta diversity of the invertebrate assemblages between time periods was tested using the betadisper() function of the vegan package. The envfit function in the vegan package was used to test the relationships between catchment variables with the NMDS ordination axes. Significance of the variables was tested using 999 permutations and significant variables (p < 0.05) were then projected onto the NMDS ordination.

Using Bray-Curtis dissimilarities calculated for each site between the 1991-1995 and 2015-2019 data, linear regressions were performed using the lm function to analyse the relationship between temporal change in the macroinvertebrate community and arable and grassland change. Land use change was calculated for each site's catchment between 1990 and 2015 using the land cover change dataset. This was to test Hypothesis 5, that site dissimilarity from the start to the end of the time series will be greater where shifts to arable agriculture have occurred.

## 5.3.6 Community Weighted Mean (CWM) trait change through time

Annual estimates for community weighted mean traits were calculated and plotted with a loess smoother to visualise changes in functional composition through time. Linear regressions were then used to investigate the relationship between CWM traits with site catchment improved grassland and arable land cover (%) from the 2007 land use map, to test Hypothesis 4. Mean CWM values for each site/trait were calculated using data from all years, with linear regressions performed using the lm function.

## 5.4 Results

## 5.4.1 Overall trends in invertebrate assemblages in the Wye

Macroinvertebrate richness increased by 14 % over the period 1992-2018; equivalent to a gain of 2.8 taxa on average (19.5 to 22.3 taxa; Figure 5.4a). The values for the first and last years for the smoothed time series were removed to increase the robustness of the estimate (Hewson and Noble 2009). Richness fluctuated around the trends, with peaks in 1996, 2005 and 2011 which were followed by troughs in 2002, 2008 and 2013 and multiple significant turning points (Figure 5.4a). Following 2013, richness increased until the end of the time series where it reached its highest point. Functional richness saw similar trends to richness (Figure 5.4b). Three peaks and troughs in functional richness occurred, with just one peak and trough where these were not identical to richness, and just one year apart in these instances. Functional richness increased by 13 % in the years 1992-2018.

CA1 remained relatively constant in the first half of the time series, with a slight increase until 1997 before a slight decline until 2005 (Figure 5.4b). Subsequently CA1 score largely increased, typical of greater EPT prevalence until 2013. Following this it decreased to below its starting value, reflected by a decline of 0.04 from 1992 to 2018. FCA1 showed the most consistent trend across the time series, with the fewest significant turning points (Figure 5.4d). It increased from the beginning of the time series until 1999, reflecting increases in taxa with a greater prevalence of medium body sizes, preferences for faster velocities and coarser substrate, and feeding strategies of deposit feeding, shredding, and scraping. FCA1 score then declined until 2004, reflecting taxa with preferences for lower current velocities, with large or smaller sizes, with parasitic, piercing and filter feeding habits (Appendix D Table D.1). FCA1 then increased before reaching its highest point in 2013, followed by a decline. The overall trends were similar to CA1 score, although declines were not as great, leading to an increase of 0.04 from 1992 to 2018.



Figure 5.4 Invertebrate trends for the river Wye in the years 1991-2019, represented by (a) invertebrate family richness, (b) functional richness, (c) CA1 scores, and (d) fuzzy CA1 scores. Estimate displayed as solid black line, with dotted lines and shaded area indicating 95 % confidence limits. Coloured dots along the smoothed line represent statistically significant inflections in the gradient of the curve: red indicating reduced rate of increase or greater rate of decline, and blue indicting greater rate of increase or reduced rate of decrease.

#### 5.4.2 Drivers of invertebrate trends

The environmental covariates explained 27–68 % of the variation in macroinvertebrate communities (Table 5.1). CA1 was the most predictable (adjusted  $r^2$  = 0.68), followed by FCA1 score (adjusted  $r^2$  = 0.54). Richness was less predictable (adjusted  $r^2$  =0.34), followed by functional richness which was least predictable (adjusted  $r^2$  = 0.27).

There was little evidence that temporal variation in the invertebrate community could be accounted for by water quality, temperature or discharge from the findings of GAMM analysis. Of all invertebrate measures, just CA1 score was significantly related to any of these measures, specifically discharge (Table 5.1).

Taxonomic richness was only significantly related to catchment improved grassland ( $p \le 0.001$ ), with no relationship to water quality or discharge variables. Richness increased until around 25 % catchment improved grassland where the trend plateaued before decreasing after around 75 % catchment land use (Table 5.1; Figure 5.5). Functional richness was only significantly related to catchment improved grassland ( $p \le 0.05$ ), following a similar inverse U-shaped relationship as the relationship of richness and improved grassland. Functional richness increased until just after 50 % catchment improved grassland, before decreasing after this point.

The first correspondence analysis axis score (CA1 score) analysis was significantly related to discharge, catchment arable land and improved grassland (Table 5.1). CA1 decreased with increasing catchment arable and improved grassland cover, and increased with discharge (Figure 5.5). FCA1 was only significantly related to catchment arable land use ( $p \le 0.001$ ), decreasing with increasing catchment arable land cover (Figure 5.5). Taken together this indicated a relative loss of EPT taxa and shift towards taxa with preferences for lower current velocities, with large or small (cf. intermediate) body sizes, and with parasitic, piercing or filter feeding habits (Appendix D Table D.1) as catchments became more agricultural.



Figure 5.5 Smoothed predicted responses of macroinvertebrate CA1 scores, richness, fuzzy correspondence analysis axis and functional richness from generalised additive mixed models (GAMMs) for each significant term. Dotted lines denote ± standard error. All variables were held at their median for plotting, discharge units are deviates.

Table 5.1 Generalised additive mixed model outputs for CA1 score, richness, FCA1 and functional richness, with smoothed terms for water quality, discharge and catchment arable and improved grassland cover. Bold text indicates significant p – values (<0.05). Effective degrees of freedom (edf) is an indicator of the degree of nonlinearity: 1 = linear, with higher values indicating increasing nonlinearity.

		(	A1		Richness				FCA1				Functional richness			
	Smoothed terms				Smoothed terms				Smoothed terms				Smoothed terms			
Variables	edf		F	р	edf		F	р	edf		F	р	edf		F	р
рН	1	0.01	9	0.091	1	0.45	5	0.501	1	0.023	3	0.881	1	0.00	2 0	.967
BOD	1	1.38	3	0.241	2.151	2.23	3	0.087	1	1.344	1	0.248	1	0.00	0 0	.985
Nitrate	2.406	0.50	5	0.420	1	0.38	כ	0.539	2.544	0.992	2	0.387	1	0.01	.0 0	.919
Phosphate	1	0.55	9	0.456	1	1.17	5	0.280	1	0.296	5	0.587	1	1.15	5 0	.284
Water temperature	1	2.64	5	0.106	1	2.25	4	0.135	1	0.153	3	0.696	1	2.66	51 C	.105
Discharge	1	3.96	0	0.048	1	1.34	4	0.248	1	0.139	9	0.710	1	1.32	6 0	.251
Catchment arable	1	14.8	77	<0.001	1	0.26	8	0.268	1	11.72	24	0.001	1	1.16	51 C	.283
land (%)																
Catchment	1	4.25	5	0.041	3.677	6.41	Ð	<0.001	1	0.880	)	0.350	2.252	3.93	2 0	.020
improved grassland																
(%)			_				_									
	Parametric coefficients				Parametric coefficients				Parametric coefficients				Parametric coefficients			
	Est.	Std.	t	p	Est.	Std.	t	р	Est.	Std.	t	р	Est.	Std.	t	p
	Error			Error				Error				Error				
Intercept	2.741	0.796	3.444	0.001	6.872	12.801	0.537	0.592	0.619	0.248	2.490	0.0138	-0.011	0.008	-1.391	0.166
Easting, Northing	-0.000	0.000	-3.475	0.001	0.000	0.000	1.085	0.280	0.000	0.000	-2.476	0.0143	0.000	0.000	2.263	0.025
	r <sup>2</sup> = 0.68				r <sup>2</sup> = 0.335				r <sup>2</sup> = 0.535				r <sup>2</sup> = 0.268			

#### 5.4.3 Community response to changing land cover

Non-metric multidimensional scaling showed a high degree of overlap between the invertebrate communities in the years 1991-1995 and 2015 – 2019 (Figure 5.6a). There was no evidence of homogenisation through time, as beta-diversity was not significantly different between the two time points ( $F_{1, 130} = 0.024$ , p = 0.877) (Figure 5.6a).

Ordination of invertebrate assemblage composition at each site between time periods showed erratic shifts over time (Figures 5.6b and 5.7). The highest density of samples had communities characterised by Ephemeroptera (Odontoceridae, Baetidae) and Trichoptera (Sericostomatidae, Goeridae, Hydropsychidae, Rhyacophilidae and Glossosomatidae), and many showed little change in composition through time (Figure 5.6b). The largest shifts through time were observed at higher values of NMDS axis 2, representing samples characterised by some plecopteran families and other invertebrates including beetles (Scirtidae, Dytiscidae and Noteridae), Megaloptera (Sialidae) and flatworms (Planaria) which tolerate a range of conditions. There was no obvious directional trend amongst higher NMDS2 samples, however, several sampling sites gained EPT taxa over time, while others lost these and instead gained less-sensitive taxa (e.g. Sialidae, Asellidae). At the highest values of axis 1, larger changes occurred where communities were characterised by Plecoptera (e.g. Perlidae and Chloroperlidae), these communities remained characterised by stoneflies despite larger changes through time, mostly characterised by an increasing composition of Perlidae (Figure 5.7). At the lowest values of axis 1, larger changes also occurred, where communities were composed of families typical of slower flows and lower water quality (e.g. Glossiphoniidae, Sphaeriidae, Gammaridae; Figure 5.7), but again there was no consistent direction of change overall.



Figure 5.6 a) Non-metric multidimensional scaling (NMDS) ordination plot, with convex hulls showing community structure differences between the 1991-1995 and 2015-2019. Yellow polygon contain sites from 1991-1995 and green polygon contains sites from 2015-2019. b) NMDS plot for community structure of sites, thin arrows connect 1991-1995 to 2015-2019 values for each site, showing direction of change. Stress = 0.16.



Figure 5.7 Non-metric multidimensional scaling (NMDS) species (family) scores for river invertebrate taxa. Aphel= Aphelocheiridae, Asell=Asellidae, Astac =Astacidae, Baeti=Baetidae, Brach=Brachycentridae, Caeni=Caenidae, Calop=Calopterygidae, Capni=Capniidae, Chiro=Chironomidae, Chlor=Chloroperlidae, Coena=Coenagrionidae, Cordu=Cordulegastridae, Corix=Corixidae, Dendr=Dendrocoelidae, Dryop=Dryopidae, Dytis=Dytiscidae.Noteridae, Elmid=Elmidae, Ephem=Ephemerellidae, Ephem=Ephemeridae, Erpob=Erpobdellidae, Gamma= Gammaridae, Gerri=Gerridae, Gloss=Glossiphoniidae, Goeri=Goeridae, Gyrin=Gyrinidae, Halip=Haliplidae, Hepta=Heptageniidae, Hydrob= Hydrobiidae.Bithyniidae, Hydrom=Hydrometridae, Hydroph=Hydrophilidae.Hydraenidae, Hydrops=Hydropsychidae, Hydropt=Hydroptilidae, Lepid=Lepidostomatidae, Leptoc=Leptoceridae, Leptop=Leptophlebiidae, Leuct=Leuctridae, Libel=Libellulidae, Limne=Limnephilidae, Lymna=Lymnaeidae, Mesov=Mesoveliidae, Nemou=Nemouridae, Nerit=Neritidae, Odont=Odontoceridae, Oligo=Oligochaeta, Perli=Perlidae, Perlo=Perlodidae, Philo=Philopotamidae, Physi=Physidae, Pisci=Piscicolidae, Plana=Planariidae.Dugesiidae, Plano=Planorbidae, Platy=Platycnemididae, Pleid =Pleidae, Polyc=Polycentropodidae, Potam=Potamanthidae, Psych=Psychomyiidae.Ecnomidae, Rhyac=Rhyacophilidae.Glossosomatidae, Scirt=Scirtidae, Seric=Sericostomatidae, Siali=Sialidae, Simul=Simuliidae, Siphl=Siphlonuridae, Sphae=Sphaeriidae, Taeni=Taeniopterygidae, Tipul=Tipulidae, Valva=Valvatidae.



Figure 5.8 Non-metric multidimensional scaling (NMDS) ordination plots showing (a) invertebrate community structure of sites in 1991-1995 and 2015 – 2019. (b) Envfit vectors (black segments) are overlaid on the NMDS, with each significantly correlated with the ordination axes. Arrow directions indicate the environmental gradient increasing in magnitude and arrow lengths are proportional to strength of correlation with the ordination.
A large proportion of the variation in community structure could be accounted for by stream and catchment variables (Table 5.2). The envfit analysis indicated that arable land accounted for the largest amount of variation in the invertebrate community, followed by altitude and grassland (Table 5.2, Figure 5.8). Calcareous geology in the catchment had no significant exploratory ability in this analysis ( $r^2 = 0.039$ , p = 0.073). Altitude and slope had similar relationships with community composition, as did arable land and urbanisation (Figure 5.8). Increasing arable and urbanised land, as well as BFI appeared to be more associated with Plecoptera, while urbanisation had a weaker association (Figure 5.8). Sites within this analysis had low percentages of urban land cover, ranging from 0-16 %, with a median value of 0.6 %. Larger catchments were associated with a greater prevalence of the mollusc Neritidae and Trichoptera (Psychomyiidae.Ecnomidae). Higher catchment cover of grassland was associated with a greater prevalence of the mollusc Physidae, while higher stream power was characterised by a community with Hydrometridae, Dendrocoelidae and Valvatidae (Figure 5.8). Higher altitude and slope were associated with a higher prevalence of Sialidae, although altitude had a stronger association with community composition than slope (Figure 5.8).

Environmental	Envfit	
variables	p	r <sup>2</sup>
Arable land	0.001	0.685
Urbanisation	0.001	0.232
Grassland	0.001	0.472
BFI	0.001	0.244
Catchment area	0.001	0.180
Channel slope	0.001	0.245
Altitude	0.001	0.605
Specific stream	0.001	0.410
power		
Calcareous geology	0.073	0.039

Table 5.2 Correlations between environmental variables and Non-metric multidimensional scaling (NMDS) ordination of invertebrate communities using *envfit* analysis.



Figure 5.9 Relationship between community Bray-Curtis dissimilarity (a) Arable land and (b) grassland land use change from 1990-2015, based on simple linear regression models (n=66).

Despite land use changing within the catchments of sites used in this analysis, with a mean 13 % increase in arable land cover and 5 % decline in grassland cover, there was no evidence that larger changes in the invertebrate community were observed in catchments with a higher degree of land cover change. No significant relationship was found between Bray-Curtis dissimilarity between 1991-1995 and 2015-2019 with arable land use change (adjusted  $r^2 = -0.015$ , p = 0.803; Figure 5.9a) or grassland change (adjusted  $r^2 = -0.007$ , p = 0.466; Figure 5.9b) from 1990 to 2015.

#### 5.4.4 Relationship between CWM traits and land use

Functional traits varied significantly with the extent of agriculture in the catchment. A greater proportion of the 43 CWM traits varied significantly with the extent of arable, compared to grassland, land cover: 37 significant relationships compared to 20, respectively (Figure 5.10). Significant relationships were found between arable land use and for all river velocity preferences, a significant positive relationship with invertebrates from 2-4 cm and negative relationship with invertebrates of 0.5-1 cm (Figure 5.10). The largest negative coefficient with improved grassland was for predators and most positive was with scraping feeding habit. For most traits, opposite relationships were found with arable or grassland land cover, indicating different effects upon the array of biological traits present (Figure 5.10). Slope coefficients were small for all variables, with the greatest positive and negative slope coefficient 0.0012 and -0.0010. Full plots are presented in Appendix D (Figure D.3).



Figure 5.10 Summary of results from linear regressions between community weighted mean (CWM) of invertebrate traits with arable and improved grassland (%) cover. Point size represents p-value of linear regression and colour of point signifies slope coefficient, negative = red, positive = blue and 0 = grey. Individual plots in Appendix D (Figure D.3).

### 5.5 Discussion

Assessing the biology within an aquatic system, such as its macroinvertebrate community, can give insight into the overall condition of the water body. Analyses of how communities have changed through time using multiple measures aimed to gain insight into how biota are faring within the Wye system. While the Wye is well-studied and monitored, with current focus on the physio-chemical stressors within the system (NRW, EA and Natural England, 2021; Environment Agency, 2022b), this type of analysis can illustrate how this may be affecting biota over time. Therefore, combining this analysis with investigations of how changes vary according to catchment characteristics and water quality variables, the aim was to get a more complete picture of invertebrate community changes within the Wye in recent years. Illustration of fish population data for example focusses on individual species, opposed to entire community changes (Clews *et al.* 2010; Environment Agency 2021b). Analysis of invertebrate data are limited, meaning this research will contribute to filling knowledge and evidence gaps for the Wye (Environment Agency 2022b).

In line with Hypothesis 1 and 2, results indicate that trends through time were variable for different invertebrate measures within the river Wye. Sampling sites were distributed across the river Wye catchment, covering upland and lowland areas, and a mix of land use types (Figure 5.2 and 5.3). These trends are therefore superficially, but not completely, representative of the Wye as a whole, as bias was not reduced using a similar poststratification procedure as used in Chapter 3. Macroinvertebrate richness increased by 14 % throughout the time series (1992-2018), while fluctuating around the trend, similar in nature to overall CA1 score trends for England and Wales (Chapter 3). Climatic variability has been hypothesised and demonstrated to drive these types of cyclical trends in other work (Bradley and Ormerod 2001; Vaughan and Ormerod 2014c). No relationship was, however, found between any invertebrate measure and discharge or water temperature – suggesting that this may not be the case in this instance. While in Chapter 3, functional richness did not increase to the same degree as richness, implying a reduction in niche breadth, functional richness in the Wye increased by 13 % through time (1992-2018). These results which indicate biological recovery are at odds with the current concerns surrounding the condition of the Wye. Despite this, declines could have occurred in the years beyond this study. In

addition, the status of the Wye is known to vary across space (Jarvie *et al.* 2003), which means these analyses may be missing localised severe declines, as trends were not disaggregated spatially.

From the start to the end of time series CA1 score declined slightly, indicating that communities were composed of fewer sensitive taxa and more pollution tolerant taxa by 2018/19. This supports results from Chapter 4 which found a decline in CA1 score in parts of Wales corresponding to the Wye. This decline was not consistent, as CA1 score fluctuated over the years (reaching its peak at the end of 2013 before declining). Change in CA1 score explains some trends in functional richness (although this would mostly be due to increases in taxonomic richness increasing the number of species present), with the turnover of some species reflected in changes in CA1 score likely leading to increased functional richness. Fluctuations in CA1 score were potentially driven by climatic variability or the dilution of some chemical determinands, as there was a weakly significant relationship with discharge. Arable land and improved grassland in particular were found to account for much of the variation in community composition, among other catchment and site level variables in NMDS analysis. Considering the well-established impacts of land use on water quality and ecosystems, it would be expected that these would be reflected in the invertebrate communities within the Wye (Allan 2004; Crooks et al. 2021). Land use within the Wye has changed considerably throughout the years 1991-2019; 24 % of land cover in the Wye changed in the years 1990-2015, including a 7 % shift to arable and 6 % shift to improved grassland (Rowland et al. 2020). This is accompanied by the reported increase in intensive poultry units (Morfett, 2022). Despite Hypothesis 5, community-dissimilarity within sites was not found to differ with catchment arable and grassland cover change. This implies that these types of land cover change were not responsible for the changes in communities. Despite this, it is plausible that some land use changes were poorly captured by the land cover change map due to their small footprints (e.g. increases in intensive poultry units).

Despite a slight increase from the start to the end of the time series, functional composition (fuzzy CA1 score) showed similar overall trends as CA1 score, sharing some significant turning points (although more were observed for functional composition). Functional composition reflected a range of traits, some of which capture the ways in which organisms interact with the environment (e.g. feeding habits and food). Therefore, changes in

functional composition may alter ecosystem functioning, potentially changing provisions of ecosystem services (Covich *et al.* 1999; Raitif *et al.* 2019). For example, functional composition reflected by few shredders may reduce leaf litter decomposition which can alter nutrient cycling (Wallace and Webster 1996; Raposeiro *et al.* 2018). Changes in individual traits would be expected to match closely changes in taxa, with shifts away from species typical of faster flowing, better quality water (e.g. Ephemeroptera, Plecoptera and Trichoptera) or slower flowing conditions with fine substrate and poorer water quality (e.g. molluscs and leeches) likely reflected in the composition of functional traits in communities. Additionally, functional composition in rivers is influenced by biotic and abiotic conditions within and adjacent to the river (Allan 2004; Yegon *et al.* 2021), similar to taxonomic composition. In this instance, while the slight declines in CA1 score indicated a shift towards taxa of poorer water quality and slower flows, FCA1 score, however, implied the opposite. Despite this, the overall increase through time (and decrease in CA1 score) was minor (0.04 in both cases).

A large proportion of the variation in macroinvertebrate communities could be attributed to a combination of water quality, discharge, and land use data ( $r^2 = 0.27 - 0.68$ ). Arable land was linked with reductions in CA1 score and functional composition, whilst CA1 score similarly declined with improved grassland cover. These results suggest that the agricultural activities within the Wye catchment led to a shift towards invertebrate communities and the prevalence of invertebrate traits typical of poorer water quality. National scale research in Chapter 3 supports this, which found that overall CA1 score was lower with greater catchment arable and improved grassland percentage cover. Arable and improved grassland influenced trait composition within the Wye, with arable land in particular being correlated with a large number of CWM traits. Agricultural activities are known to cause varying degrees of stress to rivers. This ranges from their impact on water quality through nutrient additions, increasing fine sediments, other pollutants (e.g. pesticides), as well as increases in water temperature where changes in riparian vegetation can increase light reaching the river bed (Allan 2004; Fierro *et al.* 2017).

In contrast to the decrease in CA1 score with increasing catchment improved grassland, richness and functional richness showed an inverted U-shaped relationship. Richness increased until around 25 % catchment improved grassland before remaining constant until

around 75 % before it decreased again. While functional richness increased until around 50 %, before decreasing. As improved grassland may result in less enriched runoff entering rivers compared to arable land, where tillage, exposed soils and inconsistent uptake of nutrients by plants may lead to greater leaching (Wan and Yang 2007; Milazzo *et al.* 2023), low coverage of improved grassland may indicate other more intensive land uses are present (e.g. arable land use). This may lead to richness initially increasing before the impacts of improved grassland on invertebrate richness leads to reductions in richness. In Chapter 1, richness almost consistently remained higher with greater catchment improved grassland (in contrast to CA1 score where lower values are observed with higher catchment improved grassland). Richness is also just one facet of diversity, which does not describe the communities present. Higher richness could indicate a community rich in generalists of poorer water quality, and therefore must be considered in context alongside other invertebrate measures (Gotelli and Colwell 2001; Su *et al.* 2004; Cadotte *et al.* 2011).

While agricultural land use was found to impact invertebrate communities in the Wye, water quality variables were not found to be significantly linked to changes in invertebrate communities – as hypothesised. This could be due to communities of invertebrates present in the Wye already being comprised of organisms which are tolerant to poorer water quality and its biological consequences. Discharge was the only variable apart from land cover which explained any variation in an invertebrate measure, with CA1 score increasing with discharge. Discharge is known to impact dilution of pollutants and buffer water temperature rises (Sinokrot and Gulliver 2000; Van Vliet et al. 2011; Islam et al. 2015). Structural equation models investigating relationships between water quality, land use and discharge on CA1 score in Chapter 4 found that discharge had a negative influence on phosphate concentration, BOD, and water temperature, while also having a positive relationship with CA1 score. In addition to dilution of pollutants and buffering of water temperature changes, discharge would also impact the communities present through the altering of physical conditions. Lower discharge would likely result in invertebrate communities which contribute to a lower CA1 score (Jackson, Gibbins and Soulsby, 2007; Dewson, James and Death, 2007). Dilution capacity has been found to have an impact on the water quality within the Wye, potentially explaining how agricultural impacts and discharge impacts have been found while water quality impacts are absent. The upper and mid-sections of the Wye

have historically been found to have good water quality, mainly due to their high dilution capacity, whilst the south and east of the catchment have high dilution capacity and high inputs (Jarvie *et al.* 2003).

No significant differences in the community dispersion of invertebrates in the years 1991 – 1995 and 2015 – 2019 were found, suggesting that beta-diversity of invertebrate communities did not change within the Wye between these time periods, contrary to Hypothesis 6. This complements CA1 score results which changed very little from the start to the end of the time series. As with CA1 score, it would be expected that the purported changes in land use in the Wye would have reduced the beta-diversity. Drilling into the more specific details of changes, the greatest changes in communities occurred within the communities located around the periphery of the plot – comprised of Ephemeroptera, Plecoptera and Trichoptera as well as those of poorer water conditions and slower flows (e.g. Gammaridae). Directionality of these changes through time were not consistent, which suggests that these changes do not indicate a consistent worsening/improvement of the poorest sites or best quality sites. Changes in these communities made up of the extremes of invertebrate taxa (of the poorest and best water quality) could be indicative of changes in land use, altering water quality. For example, these sites could be located in areas where the largest land use change occurred through time. Further investigation into the effects of land use changes on the change within these communities would aid this analysis.

Altitude was the greatest non land use driver of community structure, as would be expected from a catchment which contains areas of upland and lowlands (Carter *et al.* 1996). Habitat and flow will vary between uplands and lowlands, influencing the communities present. Altitude can also indicate high or low human impacts on a river, where land use may be less intensive with higher altitude and a lower human population density (Lang and Reymond 1993; Carter *et al.* 1996). In addition, within the Wye, more upland areas comprise acid and rough grassland which is less intensively managed compared to arable and improved grassland within the lowlands (Figure 1). Invertebrate family community structure was found to also be affected by arable and grassland cover, having different effects on the community structure. Both land use types are known to have different impacts on water quality, likely impacting the directionality of the effect (Ferrier *et al.* 2001). Arable land had the greatest effect on community structure, as grassland within this analysis included less intensive

grassland types – this would be expected. This would likely be intensified by the excess of phosphates added to land in the Wye catchment, including manure added to arable land with its higher tendency for runoff into waterbodies (Wan and Yang 2007; Withers *et al.* 2022; Milazzo *et al.* 2023).

## 5.6 Conclusion

Invertebrate communities within the Wye indicated no consistent decline in the years 1991-2019. The combination of invertebrate measures used to estimate change through time highlight the importance of utilising a range of measures and long time series when investigating trends through time. While taxonomic and functional richness increased overall from 1991-2019, little overall change occurred in the community composition (CA1 score) and the functional composition (while each of these measures were subject to fluctuations through time). Beta diversity did not appear to change from the beginning to the end of the time series, implying that invertebrate communities were not being homogenised through time. Agricultural land use appeared to have the greatest impacts on invertebrate communities, whilst no water quality variables were found to influence invertebrate communities. This could be explained by the methodology or absence of some variables from this analysis which are connected to land use, or by complex interconnected relationships between variables identified in Chapter 4. A particular variable which has been attributed to some of the water quality declines in the Wye but was not included within the scope of this chapter was the effects of poultry units on the invertebrate communities. Despite these having increased substantially within the last decade, there is no observable decline observed in the final third of the time series. This could indicate that these may be having no impact on the invertebrate communities up to 2019, potentially as communities were already made up of invertebrates tolerant to poorer water quality. As the effects of poultry units will not necessarily line up spatially with the locations of units, due to spreading of waste products on agricultural areas across and outside of the Wye catchment, this would be very challenging to investigate. In light of the attention that this has gained in recent years, this would be an important future research area. This chapter highlights that while investigations at the catchment scale have the potential to use finer detail to investigate biodiversity trends in rivers and identify links between catchment variables as well as water quality – this is challenging.

# Chapter 6. General discussion

# 6.1 Overview

The studies which make up this thesis aim to identify biodiversity status and trends within English and Welsh rivers, illustrating the complexity of change through space and time, and characterising the relationships between environmental variables and biological change. Outcomes of this research aim to contribute to the understanding of overall invertebrate trends, which is a topic of global interest (e.g. Baranov *et al.* 2020; van Klink *et al.* 2020; Rumschlag *et al.* 2023). Results also add to the body of evidence surrounding the status of rivers in the UK and the river Wye in particular. While the quality of rivers nationally is monitored extensively in accordance with environmental legislation, analysis of long-term trends and attributing changes to anthropogenic activities is challenging. This is due in part to changes in methodology, absence of monitoring of some pollutants and the transient nature of pollutants passing through watercourses (Whelan *et al.* 2022). Despite many rivers failing to meet water quality and ecological standards in the UK (NRW, 2021; House of Commons Environmental Audit Committee, 2022), measuring how biodiversity is changing through time can give managers and policy makers an indication of whether actions are moving the status of rivers in the right direction.

This chapter summarises the main findings within each chapter of the thesis, discussing how these interact with one another and how they feed into the wider area of research. Implications of the findings on policy are outlined, and future directions for research recommended.

# 6.2 Main findings

## 6.2.1 Chapter 2. A literature review exploring the status and threats to rivers

A comprehensive literature review was undertaken in Chapter 2, exploring the main themes of the thesis. Consistent with Hynes (1974), this highlighted that catchment land use is a central driver influencing the condition of river ecosystems, alongside changing climate and water quality. The review found that there is a large body of research about land use and its influence on many freshwater variables (e.g. water quality, hydrology) and river ecosystems. Despite this, it found limited understanding of the role land use and its interactions play in determining the condition of the UKs rivers. The review also reaffirmed the value of invertebrates as indicators of river quality. The overall aims of the thesis were set up to fill gaps in research, to identify key priorities and areas where policy and actions should be focussed.

6.2.2 Chapter 3. Long-term ecological trends in English and Welsh rivers – Evidence of recovery

Chapter 3 aimed to investigate how communities of invertebrates have changed through time, attempting the most comprehensive and least biased assessment of post-1990 national river invertebrate trends to date. This found trends in invertebrate communities through time which were consistent with an overall biological recovery of rivers in England and Wales, although it was not possible to rule out a slowing of the rate of change in the last few years. The greatest recovery occurred within urban rivers, although recovery also continued within rural areas. Whilst richness increased, this was not a consistent pattern through time, with a long period of stasis in the mid-point of the time series. By contrast, the prevalence of pollution-sensitive taxa increased far more consistently through time. This highlighted the need to consider multiple measures of invertebrate communities throughout the rest of the thesis, to obtain the fullest possible picture of change through time.

# 6.2.3 Chapter 4. Spatial patterns of change in river invertebrate communities and their potential causes

Chapter 4 revealed the extent to which temporal change in invertebrate communities varied across England and Wales, and the degree to which land use, climatic and water quality variables could account for the changes. Richness and the community composition showed contrasting geographical patterns of change, suggesting that the driving forces behind richness and composition trends differed, despite similar overall trends through time (Chapter 3). Moreover, the relationships of water quality and catchment variables with invertebrate communities were found to vary across space and through time. Structural equation models revealed interactions between catchment variables, water quality and hydrology measures and invertebrate communities. One of the main conclusions of this chapter was the complexity and context-dependency of relationships between environmental variables and invertebrate communities. This suggested that a smaller scale study, with lower environmental heterogeneity would complement the national-scale analyses. From these

results, it was determined that to investigate the relationships between environmental variables and invertebrate communities a catchment scale approach would be necessary. This was due to the directionality of relationships between water quality variables and land use with invertebrate communities clustering together at the size of or greater than a river catchment. Therefore, using a catchment scale, spatial variation in the directionality of relationships was hoped to be sufficiently low to explore relationships between drivers and invertebrate communities.

6.2.4 Chapter 5. Invertebrates in the river Wye – A case study investigating community trends, the role of land use and other environmental variables

The final data chapter utilised the river Wye as a catchment scale case study, maintaining the focus on 1991-2019. This found that taxonomic and functional richness increased through time, while the communities present and the functional composition changed little overall. Arable and improved grassland were the variables most significantly related to changes through time, and discharge was the only non-land use variable related to invertebrate changes. Temporal changes were not correlated with changing nutrient concentrations, nor were they related to changes in pH, temperature, or BOD. The majority of community composition could be accounted for by altitude and the extent of arable land and improved grassland in the catchment. There was extensive conversion of grassland to arable land in some parts of the Wye between 1990 and 2015, yet these changes were not related to the magnitude of changes in the invertebrate community. Contrary to the prevailing narrative in the media (Laville 2020; McKie 2021; Rivercide. 2021), no evidence of declining ecological condition for the catchment as a whole could be found up to 2019. The absence of any relationships between changing water chemistry and invertebrate communities was unexpected, and a range of possible explanations was discussed including omission of key chemical variables, the use of average water quality missing episodic pollution events or mismatch between the scale of analysis and impacts on invertebrate communities.

# 6.3 Synthesis

# 6.3.1 Change through time and space

With an overall picture of recovery at the national scale (Chapter 3), the findings of this thesis support much of the literature which paints a picture of overall recovery within rivers in the UK (Vaughan and Ormerod, 2012; Outhwaite *et al.* 2020), in Europe (Pilotto *et al.* 

2020), globally (van Klink *et al.* 2020) but not in the United States (Rumschlag *et al.* 2023). There were hints that recovery was slowing down in recent years, which could mean that the benefits of reductions in gross pollution are becoming exhausted. This could indicate that without additional changes to policy, management or regulation, invertebrate communities may reach a point of stasis where no further recovery occurs. Despite this, rapid changes continued in urban rivers, indicating that their recovery from gross pollution is ongoing. Additionally, there was evidence of losses of pollution sensitive taxa across much of the English and Welsh uplands, yet there was little decline in richness in these areas. This suggests that turnover has occurred in these areas, with more tolerant species replacing pollution sensitive taxa.

Locations where richness and pollution-sensitive taxa declined (Chapter 4) were positioned, for the most part, in areas of England and Wales where these measures were highest in 1991 – 1993. Indicating that declines have occurred largely in the most diverse or highest quality rivers. It has been suggested that improvements in the most degraded urban areas is leading to these "closing the gap" on sites of better water quality, which was used to explain increasing homogenisation through this time (Chapter 3). This evidence of decline amongst these highest quality sites is certainly a cause for concern, which may also be contributing to increases in homogeneity nationally.

Whilst taxonomic richness, composition and functional richness responded differently to environmental change through time, the spatial analysis in Chapter 4 illustrated the extent to which invertebrate community measures were responding differently to environmental conditions. For example, richness declined in rivers in SW Wales, NE and SW England, while pollution-sensitive taxa increased within these areas. The contrasting trends between richness and composition (CA1 score) re-emphasises the importance of using multiple measures to assess biodiversity trends, as single metrics such as taxon richness may overlook subtle changes (Larsen *et al.* 2018). Spatial results also draw attention to the limitations of using a single trendline to describe long-term changes at national scales – considerable spatial heterogeneity is ignored.

Based on spatial analysis from Chapter 4, richness in the Wye was expected to increase throughout the time series, while CA1 score was expected to remain relatively constant – due to increases in the lower Wye and decreases in the upper Wye observed at the coarse

national scale. Chapter 5 confirmed these expectations, which should increase the confidence when interpreting maps produced in Chapter 3.

6.3.2 Identifying relationships between water quality variables and invertebrate communities

The ability to identify associations of abiotic and biotic conditions with invertebrate communities is known to vary across different scales; for example a relationship that appears absent across a large scale may be observed in a subset of the overall area (Hambrook Berkman *et al.* 2010). An initial analysis for England (Pharaoh *et al.* 2021) did not find any relationships between water quality variables (pH, temperature, BOD, nitrate, phosphate) or median discharge and trends in invertebrate communities for England as a whole. Models instead were successful at explaining geographical patterns in invertebrate communities. One of the aims of Chapter 4 was therefore to test whether relationships with water quality, climate and land cover emerged at the local or regional scale, or vary through time. Geographically weighted regression confirmed the presence of large spatial variation in these relationships across the spatial extent of England and Wales, which might explain why relationships were not identified at a national scale. Relationships tended to be consistent at local or regional scales similar to the area of large river catchments. Therefore, it was expected that they would be easier to characterise by focusing on the river Wye in Chapter 5.

Chapter 5 did not, however, identify any relationships between water quality variables and changes in invertebrate communities at the catchment scale. The analysis treated water quality variables as deviates, where the mean value at each sample site was zero, so that it focused on temporal change, however strong relationships may have been found for among site differences. The only water quality or discharge variable which was found to have a significant relationship with any invertebrate measure within the Wye was a positive relationship between discharge and CA1 score. National scale analysis in Chapter 4 found that discharge had a negative influence on phosphate concentration, BOD, and water temperature, while also having a positive relationship with CA1 score. As discharge is known to dilute pollutants and buffer water temperature rises (Sinokrot and Gulliver 2000; Van Vliet *et al.* 2011; Islam *et al.* 2015), discharge would be expected to impact the communities present through altering of physical conditions. Lower discharge also would be expected to

result in invertebrate communities which contribute to a lower CA1 score (Jackson, Gibbins and Soulsby, 2007; Dewson, James and Death, 2007). The absence of relationships between any water quality measures and invertebrate communities were not, however, expected at the catchment scale. The catchment scale may have been too large to identify relationships between water quality drivers and invertebrate communities, with smaller scale reach and segment scale analysis such as that used by Hambrook Berkman *et al.* (2010) potentially more appropriate. The use of annual median water quality data and discharge likely reduced the potential for this research to identify these relationships. While these measures maximised the number of sites that could be retained, they resulted in the loss of some details, such as seasonal variations in water quality and discharge. The inclusion of the extremes of flow and quality might have explained some of the long-term trends in invertebrate communities (Pace *et al.* 2013).

#### 6.3.3 Identifying links between land use and invertebrate communities

Each of chapters 3–5 identified relationships between land use and invertebrate communities. Chapter 3 was able to confirm earlier analysis that community changes varied along urban and agricultural gradients (Vaughan and Ormerod 2012), while Chapter 4 revealed variation in the relationship between invertebrate communities and land cover across England and Wales. The effects of different land use types on invertebrate communities varied across England and Wales. This is likely partly indicative of different land management practices across the country which are contained within the same broad land cover designations, but may have different impacts on rivers. For example, arable land use in west-Wales is dominated by potato crops, while the greatest density of sugar beet is located in the east of England (England Agricultural Census, 2016; Wales Agricultural Census, 2018). Moreover, the same land management may have different impacts on invertebrate communities across space, due to interactions between land use and the landscape, for example with geology and climate variables (Gottschalk and Weingartner 1998; Jencso and McGlynn 2011; Sitterson et al. 2018). For example, some regions may be more prone to runoff due to higher rainfall or low permeability of soils. This may influence invertebrate communities differently to an area with the same land management but with lower rainfall and more permeable soils. When these findings are combined with the

relationships between land use, invertebrate communities and water quality parameters a far more complex picture is revealed.

Chapter 5 hoped to delve further into the finer details of land use impacts on invertebrate communities in the Wye, taking advantage of the finer scale. In particular, this investigated whether communities were more dissimilar where the greatest land use change (arable and grassland) had occurred. This was important to investigate because land use within the Wye is reported to have changed throughout the years of this analysis (Rowland *et al.* 2020; Morfett 2022). Community dissimilarity within sites was not, however, found to differ with catchment arable and grassland cover change. It is conceivable that some land use changes were poorly captured by the land cover change map due to their small footprints, such as increases in intensive poultry units. This was a limitation of this chapter, as links between land use and invertebrate communities are well evidenced, thus land use changes in further detail, such as utilising agricultural census data or reports of poultry units (e.g. Caffyn, 2021) was not, however, possible within the scope of this chapter. This may also be absent due to a potential time lag in the response to land cover changes.

#### 6.3.4 Policy implications

Findings from this thesis give an idea of the changing ecological status of rivers in England and Wales, which can provide evidence for decision makers of the water environment. While a recovery has largely been observed, within England and Wales, and the river Wye specifically, rivers are far from meeting acceptable standards in terms of ecology and water chemistry. In England, just 14 % or rivers currently achieve good ecological status, while in Wales this figure is 44 % (NRW, 2021; House of Commons Environmental Audit Committee, 2022). Recovery since 1990 has mostly been driven by recovery from gross pollution, with reported reductions in mean concentrations of metals and BOD, a decrease in phosphate loads from both point-and diffuse-sources, and some recovery from catchment acidification. Reductions in ammonia have also been reported, driven by the EU Urban Waste Water Treatment Directive and nitrate concentrations have levelled out due to the EU Nitrates Directive (Council of European Communities 1991; Burt *et al.* 2011; Whelan *et al.* 2022). Despite this, with emerging contaminants and increases in impacts from intensive agriculture, this is a mixed picture. Outhwaite *et al.* (2020) investigated a wider timescale,

and found evidence of declines from 1970-1990, which were followed by recovery post-1990, indicating that recoveries after 1990 are compensating for declines which occurred just 20 years before the start of this time series.

Spatial illustration of change through time will provide valuable insights to policy makers, as these highlight areas which will require further scrutiny. A particular priority should be regions which were in the best condition nationally at the start of the time series, but have declined since 1990. For example, rivers in west Wales and the south-west of England had the highest richness in the 1990s, which has declined since. Simmilarly, CA1 score in parts of mid-Wales have declined where some of the highest scores were found 30 years ago. Biodiversity declines within the best quality areas could reflect losses of more specialist species. If declines were to continue, despite recovery within poorer quality sites, this could lead to a loss of the overall diversity within England and Wales.

With the attention that the status of the river Wye has garnered in recent years, these results found no compelling evidence of declines up to 2019, which is at odds with the prevailing narrative. Despite this, there could be declines which have occurred in the years beyond this study or in particular tributaries. The spatial distribution of sites was not necessarily reflective of the Wye as a whole, and post-stratification was not attempted (cf. Chapter 3) due to the relatively small sample size. As with Chapters 3 and 4, there was no assessment of taxa beyond macroinvertebrates (e.g. diatoms or fish). Whilst invertebrates are considered to be a good indicator of river condition, they may overlook important changes in other groups. For example, Atlantic salmon *Salmo salar* have declined throughout this timeframe in the Wye (Environment Agency 2021b).

These analyses and insights would not have been possible without the extensive river monitoring conducted by the respective agencies in England and Wales. While concern around the reductions of monitoring efforts has been raised (Whelan *et al.* 2022), the combination of careful site selection for long-term sampling and post-stratification made it possible to overcome potential limitations in this analysis. Further declines in sampling efforts do, however, have the potential to restrict future study. With the wide range of work which has provided valuable insights from these data in recent years (Dunbar *et al.* 2010; Orr *et al.* 2015; Whelan *et al.* 2022; Powell *et al.* 2023), this would leave a huge evidence gap for informing policy to safeguard freshwaters.

# 6.4 Future directions

This thesis leaves some questions unanswered which would benefit from further research. Firstly, while this thesis was successful at identifying the role of land use in invertebrate trends through time nationally and at a catchment scale, it did not investigate the impacts of land use changes, whether existing or future, on invertebrate communities at the national scale. As land use was identified as having such a strong influence on invertebrate communities, it would be expected that any changes would influence invertebrate communities. With land use known to have changed over the course of the years included in this study, this would be an important avenue to investigate (Rowland *et al.* 2020), particularly where land use is likely to change further across the UK to meet the changing needs of the population, and unfolding climate crisis (Knox *et al.* 2010). This can highlight areas which should be factored in future planning actions, as demonstrated by Bussi *et al.* (2016, 2018).

Work in this thesis was unable to identify a role for water quality variables, averaged annually, in driving invertebrate trends, even at the catchment scale. This is important, because where targets are set for concentrations of water quality variables, identifying the role that water quality variables and their causal drivers (e.g. land use) are playing in driving biodiversity trends will support this. The GWR results from Chapter 4 and limited success of Chapter 5 should be used to identify the scale at which such an analysis would be successful. Potentially, this analysis may be limited due to the absence of episodic events illustrated in these data (e.g. low flow or high flow and high surface runoff) due to the use of annual medians based on monthly measurements. These coarse measures, although important for maximising the number of sites which could be used in this analysis, are a limitation of this research due to the loss of this detail. There were some water quality variables absent in this analysis, partly because these were absent or less consistently measured in national scale monitoring data. As mentioned in Chapter 5, it may be the absence of these water determinants that has resulted in the strong relationships identified between land use variables but absence of relationships found for water quality determinands. In particular suspended sediment was not included within this analysis but has a major influence on invertebrate communities (Wagenhoff et al. 2011; Lemm and Feld 2017; Davis et al. 2018),

alongside emerging contaminants which have been increasing through time and should be included for brevity (Markert *et al.* 2022; Whelan *et al.* 2022).

With agricultural land shown to influence river biodiversity within this study and its domination within the landscape of England and Wales, any changes to land management activities will have the potential to influence river biodiversity. With recent changes to the political landscape in the UK due to Brexit, countries within the UK are parting from the widely criticised EU Common Agricultural Policy (Pe'er *et al.* 2020). With the replacement of the Basic Payment Scheme by each country with new schemes, there is a potential for agricultural impacts on the environment to change. In England, a new Environmental Land Management Scheme is to be introduced which will include paying famers to deliver environmental goods and services alongside food production (DEFRA 2023). This has the potential to be a turning point for the way in which land is managed, which could have implications for the condition of rivers and the wider landscape. Therefore, an updated analysis of long-term trends within river communities in future could be valuable to measure how this may influence river condition. As well, with different schemes implemented for each country, it would be interesting to compare how this differs between countries.

#### 6.5 Thesis conclusion

The status of river ecosystems is ever changing. Across England and Wales, the years 1991 – 2019 witnessed an apparent continuation of recovery from historical pollution. This is perhaps surprising, given the media narrative about declines and degradation within the water environment in the UK. This thesis concludes that despite recovery from gross pollution driven by some successful policies and industrial decline, there are notable exceptions such as the uplands and parts of south west England and Wales. The poor status of rivers outlined by the statutory agencies of England and Wales also signal the new and ongoing challenges within rivers, and indicates that ecological recovery is not complete. These findings therefore should not be used as a licence to disregard failings in water policy. A recovery from a very low baseline in the late 20<sup>th</sup> century is still not a picture of healthy rivers. Ongoing monitoring and analysis of the condition of rivers is essential to fulfil evidence needs to ensure the safeguarding and ongoing recovery of rivers for this and future generations.

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## Appendix B

Develue es elide e	Cinhlenuridee	Maaavaliidaa	
Dendrocoelidae	Siphionuridae	Wiesoveilidae	Нудгорпіїдае
Planariidae/Dugesiidae	Baetidae	Hydrometridae	Rhyacophilidae/Glossosomatidae
Neritidae	Heptageniidae	Gerridae	Philopotamidae
Viviparidae	Leptophlebiidae	Nepidae	Polycentropodidae
Valvatidae	Potamanthidae	Naucoridae	Hydropsychidae
Hydrobiidae/Bithyniidae	Ephemeridae	Aphelocheiridae	Psychomyiidae/Ecnomidae
Physidae	Ephemerellidae	Notonectidae	Phryganeidae
Lymnaeidae	Caenidae	Pleidae*	Brachycentridae
Planorbidae	Taeniopterygidae	Corixidae	Lepidostomatidae
Unionidae	Nemouridae	Haliplidae	Limnephilidae
Sphaeriidae	Leuctridae	Paelobiidae*	Goeridae
Oligochaeta*	Capniidae	Gyrinidae	Beraeidae
Piscicolidae	Perlodidae	Dytiscidae/Noteridae	Sericostomatidae
Glossiphoniidae	Perlidae	Hydrophilidae/Hydraenidae	Odontoceridae
Hirudinidae*	Chloroperlidae	Scirtidae	Molannidae
Erpobdellidae	Platycnemididae	Dryopidae	Leptoceridae
Astacidae	Coenagrionidae	Elmidae	Tipulidae
Asellidae	Calopterygidae	Sialidae	Simuliidae*
Corophiidae	Cordulegastridae		Chironomidae
Gammaridae	Aeshnidae		
	Libellulidae		

Table B.1 The 78 families and composite groups included in the study, following Vaughan & Ormerod (2014). Taxa marked \* were excluded from the analysis of functional richness.

Feeding habits	Substrate (preferendum)
Absorber	Flags/boulders/cobbles/pebbles
Deposit feeder	Gravel
Shredder	Sand
Scraper	Silt
Filter-feeder	Macrophytes
Piercer (plants or animals)	Microphytes
Predator (carver/engulfer/swallower)	Twigs/roots
Parasite	Organic detritus/litter
Locomotion and substrate relation	Mud
Flier	Current velocity (preferendum)
Surface swimmer	< 5 cm.s <sup>-1</sup>
Full water swimmer	5-25cm.s <sup>-1</sup>
Crawler	25-50 cm.s <sup>-1</sup>
Burrower (epibenthic)	> 50 cm.s <sup>-1</sup>
Interstitial (endobenthic)	Food
Temporarily attached	Fine sediment + microorganisms
Permanently attached	Fine detritus (≤ 1mm)
Maximal potential size	Dead plant (> 1mm)
<= 0.25 cm	Living microphytes
> 0.25 - 0.5 cm	Living macrophytes
> 0.5 - 1 cm	Dead animal (> 1mm)
> 1 - 2 cm	Living microinvertebrates
> 2 - 4 cm	Living macroinvertebrates
> 4 - 8 cm	Vertebrates
> 8 cm	

Table B.2 Traits according to Tachet used for calculation of functional richness.



Figure B.1 Density plots comparing the topographical distribution of invertebrate biology sites sampled in each year of the study, and distribution of English and Welsh sites sampled during the second RHS Baseline (dotted line). Density plots calculated for each year of the study, and RHS second baseline sites according to (a) altitude, (b) catchment area, (c) distance downstream from the source, (d) channel slope, (e) percentage of catchment attributed to urban land cover and (f) number of invertebrate samples represented per year of data.



(a)



Figure B.2 Correspondence analysis loadings for the 78 composite and family groups used in this study: (a) using presence-absence data (CA1PA), and (b) using abundance data (CA1Ab).



Figure B.3 Smoothed invertebrate trends for England and Wales in the years 1991-2019, represented by CA1 scores from abundance and data. Post-stratified estimate displayed as solid black line; dotted line and shaded area indicating bootstrapped 95% confidence limits. Dots along the line representing statistically significant inflections in the gradient of the curve: red indicating reduced rate of increase or greater rate of decline, and blue indicting greater rate of increase or reduced rate of decrease. Black points denote annual (unsmoothed) point estimates.



Figure B.4 Smoothed time series trends with (black lines) and without (red lines) poststratification weighting for (a) taxonomic richness and (b) presence-absence CA1 scores (CA1PA).



Figure B.5 Temporal trends of richness and CA1 score from presence-absence data among sites with the lowest 1% (amber) and highest 1% (green) catchment urbanisation (0 and 84%), improved grassland (0 and 100%), and arable (0 and 100%) land cover. The lowest 10% (amber) and highest 10% (green) altitude (<=10m and >=142m) and catchment area (<=9 km2 and >=327 km2). Dashed lines indicate estimates, with coloured areas indicating ± standard error.

## Appendix C

	med.pH	med.Temp	med.BOD	med.Nitrate	med.Phosphate	scaled.med.dischar	Arable.2007	Imp.grass.2007	Urbanisation.2007	_ 1
med.pH	1			0.32		-0.44	0.44			- 08
me	ed.Temp	1	0.1	0.2	0.23	-0.2	0.14	-0.15	0.2	- 0.6
	m	ed.BOD	1	0.2	0.46	-0.17	-0.09	0.0	0.4	- 0.4
	med.Nitrate 1					-0.46	0.65	-0.43		- 0.2
	med.Phosphate 1					-0.2	0.13	-0.17	0.19	- 0
			scal	led.med.d	lischarge	1	-0.51	0.22		0.2
					Ara	ble.2007	1	-0.7	-0.29	0.4
						Imp.gra	ss.2007	1	-0.16	0.6
						d	Urbanisati	on.2007	1	0.8

Figure C.1 Correlation coefficients for GWR data. Abbreviations med = median., and Imp = improved.

	Hd.pH	med.Temp	med.BOD	med.Nitrate	med.Phosphate	scaled.med.discharge	Arable.2007	Imp.grass.2007	Urbanisation.2007	
med.pH	1			0.35		-0.45	0.46			
me	d.Temp	1		0.21	0.23	-0.21			0.2	
	m	ed.BOD	1	0.21	0.46	-0.21	-0,07		0.41	
	med.Nitrate 1 0.52					-0.45	0.64	-0.41		te (
			med.Ph	osphate	1		0.15	-0.18	0.21	8 <b>9</b> 0
			scale	d.med.di	scharge	1	-0.5	0.22	-0.09	
					Arat	ble.2007	1	-0.66	-0.28	
						Imp.gra	ss.2007	1	-0.16	6 <mark>-</mark> 61
						L	Irbanisati	on.2007	1	

Figure C.2 Correlation coefficients for SEM data. Abbreviations med = median, and Imp = improved.

Table C.1 GWR bestNormalize transformations	;
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Variable	1991-2004	2005-2019
рН	orderNorm	Standardized exp(x)
Temperature	Standardized Box Cox	orderNorm
BOD	orderNorm	orderNorm
Nitrate	Standardized Yeo-	Standardized sqrt(x + a)
	Johnson	
Phosphate	Standardized Box Cox	orderNorm
Improved grassland %	orderNorm	orderNorm
Arable %	orderNorm	orderNorm
Urbanisation %	Standardized asinh(x)	Standardized sqrt(x + a)

Table C.2 SEM bestNormalize transformations

Variable	Transformation
рН	1/(max(x + 1) – x))
Temperature	none
BOD	Log(x)
Nitrate	Sqrt(x)
Phosphate	Sqrt(x)
Scaled median discharge	Sqrt
Improved grassland %	Sqrt
Arable %	log10(max(x+1)-x)
Urbanisation %	Log1p()



Figure C.3 Distribution maps of (a) Uplands, land above or below 250 m, (b) Urbanisation, (c) arable, (d) Improved grassland from 2007 land cover maps and (e) chalk streams from © Environment Agency copyright and/or database right 2015. All rights reserved. Contains Ordnance Survey data © Crown copyright and database right 2004.

Richness



Figure C.4 Local r<sup>2</sup> for GWR for explanatory power of water quality and land use in predicting invertebrate richness in (a) 1991-2004, (b) 2005-2019 and CA1 score in (c) 1991-2004 and (d) 2004-2019.

The explanatory power of water quality and land use varied widely across the geographical extent of England and Wales. Local  $r^2$  values ranged from 0.28 to 0.74 for richness, with the highest explanatory power located in regions in the north and south of England (Figure C.4a) and south-west of Wales in the first half of the time series (1991-2004; Figure C.4b).  $r^2$  was lowest in an area of the south-east of England in 2005-2019 (Figure C.4b). Explanatory power was more variable for CA1, ranging from 0.17 to 0.85; sites with the highest  $r^2$  were located in the more western areas of England and Wales, whilst this is lowest towards the east (Figure C.4c and C.4d). This remained consistent across both halves of the time series.









Figure C.5. Kriged river water quality and temperature for 1991-2004, 2005-2019 and change from 1991-2004 to 2005-2019 calculated from kriged maps for (a) phosphate, (b) nitrate, (c) BOD, (d) temperature and (e) pH.

	Rich	ness	CA1 score			
	1991-2004	2005-2019	1991-2004	2005-2019		
BOD	-1.40	-0.81	-0.09	-0.06		
рН	0.72	0.62	-0.02	-0.01		
Nitrate	-0.59	0.34	-0.00	-0.06		
Phosphate	-0.22	-0.68	-0.08	-0.04		
Temperature	-0.12	-0.11	-0.02	-0.06		
Arable	2.83	1.18	-0.12	-0.12		
Improved grassland	1.99	0.85	0.00	-0.02		
Urbanisation	-1.25	-1.72	-0.09	-0.10		

Table C.3 Mean local regression coefficient for 1991-2004 and 2005-2019 for richness and CA1 score coefficients for water quality, water temperature and catchment land use.



Figure C.6 The distribution of GWR coefficients from the richness models at the two time points (T1 = 1991-93; T2 = 2017-19).



Figure C.7 The distribution of GWR coefficients from the CA1 score models at the two time points (T1 = 1991-93; T2 = 2017-19).

## Appendix D

Table D.1 CA1 scores and NMDS axis 1 and 2 calculated for 78 and 66 families respectively. Bold names are families used for calculation of functional richness.

Family	NMDS1	NMDS2	CA1	Family	NMDS1	NMDS2	CA1
Aeshnidae	NA	NA	4.839	Leptophlebiidae	0.098	0.148	0.108
Aphelocheiridae	0.013	-1.029	-0.835	Leuctridae	-0.540	0.003	1.660
Asellidae	0.767	0.231	-1.693	Libellulidae	1.793	-0.388	-4.664
Astacidae	-0.157	-0.165	0.320	Limnephilidae	0.210	0.117	-0.196
Baetidae	-0.110	-0.027	0.132	Lymnaeidae	0.466	-0.058	-1.476
Beraeidae	NA	NA	-1.443	Mesoveliidae	0.025	-0.249	-0.395
Brachycentridae	-0.245	-1.156	-0.624	Molannidae	NA	NA	-2.606
Caenidae	-0.218	-0.475	0.409	Nemouridae	-0.367	0.377	1.283
Calopterygidae	0.576	-0.723	-2.439	Neritidae	0.305	-0.758	-1.141
Capniidae	-0.039	0.590	1.239	Notonectidae	NA	NA	-1.828
Chironomidae	-0.016	0.072	0.026	Odontoceridae	-0.238	-0.281	1.108
Chloroperlidae	-0.740	0.278	2.266	Oligochaeta	0.094	0.106	0.003
Coenagrionidae	1.616	0.431	-4.204	Paelobiidae	NA	NA	-5.725
Cordulegastridae	-1.392	1.917	3.083	Perlidae	-0.599	-0.173	1.950
Corixidae	1.188	0.312	-3.468	Perlodidae	-0.545	0.037	1.787
Dendrocoelidae	1.028	0.181	-2.018	Philopotamidae	-0.415	0.148	1.749
Dryopidae	0.308	-0.457	-0.446	Phryganeidae	NA	NA	3.281
Dytiscidae.Noteridae	0.052	0.278	-0.153	Physidae	1.309	-0.200	-3.016
Elmidae	-0.104	-0.061	0.263	Piscicolidae	0.479	-0.294	-1.885
Ephemerellidae	-0.187	-0.284	0.079	Planariidae.Dugesiidae	0.183	0.138	-0.557
Ephemeridae	0.122	-0.238	-0.576	Planorbidae	0.307	-0.042	-0.750
Erpobdellidae	0.413	-0.020	-0.976	Platycnemididae	-0.374	-0.629	-2.271
Gammaridae	0.257	0.015	-0.466	Pleidae	-0.150	0.076	-1.811
Gerridae	-0.641	0.964	1.022	Polycentropodidae	-0.469	0.115	1.097
Glossiphoniidae	0.586	0.084	-1.596	Potamanthidae	-0.025	-1.180	-0.698
Goeridae	-0.073	-0.168	0.278	Psychomyiidae.Ecnomida e	0.104	-0.589	-0.726
Gyrinidae	-0.299	-0.297	1.079	Rhyacophilidae.Glossoso matidae	-0.198	-0.074	0.697
Haliplidae	0.805	-0.037	-2.821	Scirtidae	0.027	0.144	-0.008
Heptageniidae	NA	NA	0.947	Sericostomatidae	-0.108	-0.211	0.206
Hirudinidae	-0.315	-0.146	0.683	Sialidae	0.738	0.340	-2.519
Hydrobiidae.Bithyniidae	0.394	-0.072	-0.799	Simuliidae	-0.057	-0.121	0.165

Hydrometridae	1.123	0.230	-1.276	Siphlonuridae	-1.753	1.246	4.091
Hydrophilidae.Hydraeni dae	-0.333	-0.074	0.976	Sphaeriidae	0.580	0.015	-1.088
Hydropsychidae	-0.269	-0.167	0.671	Taeniopterygidae	-0.567	0.144	1.844
Hydroptilidae	0.009	-0.331	-0.276	Tipulidae	-0.028	-0.016	0.094
Lepidostomatidae	-0.334	-0.459	0.845	Unionidae	NA	NA	-3.669
Leptoceridae	-0.080	-0.359	-0.108	Valvatidae	1.200	-0.007	-3.051

Feeding habits	FCA1	Substrate (preferendum)	FCA1
	correlation		correlatio
			n
Absorber	NA	Flags/boulders/cobbles/pebbles	0.522893
Deposit feeder	1.359758	Gravel	0.115066
Shredder	0.605148	Sand	0.172228
Scraper	0.483529	Silt	-0.67947
Filter-feeder	-1.1454	Macrophytes	-0.52788
Piercer (plants or animals)	-3.30547	Microphytes	-2.26108
Predator (carver/engulfer/	-0.8562	Twigs/roots	0.903185
swallower)			
Parasite	-5.87478	Organic detritus/litter	-0.29373
Locomotion and substrate re	lation	Mud	NA
Flier	0.335874	Current velocity (preferendum)	
Surface swimmer	-2.64557	< 5 cm.s-1	-2.04678
Full water swimmer	-0.04277	5-25cm.s-1	-0.44876
Crawler	0.251337	25-50 cm.s-1	0.88715
Burrower (epibenthic)	-1.26831	> 50 cm.s-1	1.662651
Interstitial (endobenthic)	0.429251	Food	
Temporarily attached	0.070107	Fine sediment + microorganisms	2.445239
Permanently attached	0.408348	Fine detritus (≤ 1mm)	0.768235
Maximal potential size		Dead plant (> 1mm)	0.143128
<= 0.25 cm	-2.46657	Living microphytes	0.530424
> 0.25 - 0.5 cm	-0.23774	Living macrophytes	-0.2683
> 0.5 - 1 cm	0.93068	Dead animal (> 1mm)	-1.7011
> 1 - 2 cm	0.389949	Living microinvertebrates	-0.25036
> 2 - 4 cm	-2.00828	Living macroinvertebrates	-0.73694
> 4 - 8 cm	-2.96787	Vertebrates	-7.87216
> 8 cm	-4.37133		

Table D.2 Traits according to Tachet used for calculation of functional richness with adjacent first axis of the fuzzy correspondence analysis (FCA1).


Figure D.1 Trends in individual community weighted mean traits. Individual, annual estimates are shown (blue points) with a loess smoother (black line) added to help visualise trends.



Figure D.1 Linear regressions of mean CWM trait values for each site with catchment improved grassland (%).



Figure D.2 Linear regressions of mean CWM trait values for each site with catchment arable land (%).

Feeding habitats: dpf = deposit feeder, shr = shredder, scr = scraper, fif = filter feeder, pie = piercer, pre = predator, par = parasite

Velocity preferences: > 50 cm s<sup>-1</sup>, medium = 25-50 cm s<sup>-1</sup>, slow = 5-25 cm s<sup>-1</sup>, null = < 5 cm s<sup>-1</sup>

Food: mior = fine sediment and microorganisms, detl1 = fine detritus, dpg1 = dead plant material, limi = living microphytes, lima = living macrophytes, dag1 = dead animal material, lmic = living microinvertebrates, lmac = living macroinvertebrates, vert = vertebrates. 'Absorber' was not recorded amongst the 73 taxa.

Substratum preferences: fbcp = flags/boulders/cobbles/pebbles, grvl = gravel, sand = sand, silt = silt, macp = macrophytes, micp = microphytes, twro = twigs/roots, odli = organic detritus/litter, mud = mud.

Locomotion and substratum relation: flr = flier, ssw = surface swimmer, wsw = full water swimmer, crw = crawler, bur = burrower, int = interstitial, tat = temporarily attached, pat = permanently attached.

Maximum size: less.025 =  $\leq 0.25$  cm, 025.0.50 = > 0.25-0.5 cm, 50.100 = > 0.5-1 cm, 100.200 = > 1-2 cm, 200.400 = > 2-4 cm, 400.800 = > 4-8 cm, over.800 = > 8 cm.